N-terminal matrix (MA)³ protein domain, which, in a 120-nm sphere, gives Gag a concentration of ~4.4 mM, with a crude estimate that the Gag molecules occupy 50–60% of the volume of the sphere (4). There are also ~120 Gag-Pro-Pol molecules (5). The embedded protease (PR) must dimerize, release itself from the Gag-Pro-Pol precursor, and then cleave the other PR cleavage sites in Gag and Gag-Pro-Pol (6). From these cleaved products, the nucleocapsid (NC) condenses and stabilizes the viral dimeric RNA, and ~1500 copies of the processed capsid (CA) protein reform to make the mature conical capsid structure around viral RNA to create an infectious particle (7). In this minireview, we examine outstanding issues surrounding the HIV-1 PR, the role of protein processing and rearrangement in the assembly pathway, the impact of PR inhibitor resistance on viral fitness and assembly, and the fact that all of this biochemistry takes place within the confines of a particle that is only 120 nm wide.

A Closed System

The activity of all of the viral enzymes appears to take place within a closed system, with a finite number of protein molecules available for each process. This is true for protein processing during the production of the virus particle and for viral DNA synthesis after the particle infects the next cell. Modest changes in the number of one of the viral enzymes or the number of active molecules can have surprising effects on particle assembly, maturation, and infectivity, and from this, we can infer that certain steps in replication require more than one molecule (or molecular complex) of an enzyme, whereas others require only one. The number of active enzyme molecules in a virus particle can be manipulated by titrating in an inhibitor, titrating in an inactive subunit through phenotypic mixing, or reducing enzymatic activity with mutations that confer a fitness loss. Furthermore, reductions in PR activity can have pleiotropic effects because the PR is responsible for cleaving the Gag-Pro-Pol precursor to generate active reverse transcriptase (RT) and integrase (IN). It is now clear that for replication steps that require multiple copies of an enzyme, the partial loss of enzymatic activity, to the point where this activity is limiting for replication, results in a virus particle that has enhanced sensitivity to further inhibition by an inhibitor.

The simplest example is the sensitivity of RT to non-nucleoside RT inhibitors (NNRTIs). When RT activity is partially inhibited by including an intermediate level of an NNRTI (8–10), by creating a mixture of wild-type and mutant RT subunits (8, 11), or by reducing PR activity to decrease the amount of processed RT subunits (12, 13), the remaining viral infectivity is hypersensitive to inhibition by adding additional NNRTI. The interpretation is that viral DNA synthesis requires more

³ The abbreviations used are: MA, matrix; PR, protease; NC, nucleocapsid; RT, reverse transcriptase; IN, integrase; NNRTI, non-nucleoside RT inhibitor; PI, protease inhibitor; NRTI, nucleoside/nucleotide RT inhibitor; AZT, azidothymidine; SP1, spacer peptide 1; SP2, spacer peptide 2; CA, capsid; CAI, capsid assembly inhibitor; CTD, C-terminal domain; NTD, N-terminal domain; BD, benzodiazepine; BM, benzimidazole.
than one RT heterodimer to be successful; as the number of RT molecules in the replication complex decreases, the probability of successfully completing DNA synthesis is reduced, i.e. it is easier to get to the threshold of too little RT if some of it is already missing. Thus, multiple RT heterodimers must be associated with the replication complex where viral DNA synthesis occurs in a newly infected cell, and DNA synthesis requires the participation of multiple RT complexes, making the process distributive.

The same phenomenon is seen with PR activity in the formation of an infectious particle. Partial reduction of PR activity through titration with an inhibitor (10), by mixing with an inactive subunit (11, 13), or by incorporating mutations that reduce enzymatic activity/fitness (13) results in enhanced sensitivity to further inhibition with a protease inhibitor (PI). Thus, the maturation process requires multiple PR dimers, and as the total number of active PR dimers in the assembling virion decreases, it becomes easier to titrate the remaining activity to reach a threshold of too little enzymatic activity to make an infectious particle.

The opposite phenomenon is seen with the viral IN tetramer, where reduction in IN activity through partial titration with an inhibitor (10) or including an inactive subunit (11) does not enhance sensitivity to further inhibition, presumably due to the fact that a single IN tetramer binds to the ends of viral DNA and does not exchange with free IN within the replication complex even if the bound form is inactive. Also, chain-terminating nucleoside/nucleotide RT inhibitors (NRTIs) cap the growing DNA chain instead of inhibiting RT itself; thus, viral infectivity does not become increasingly sensitive to NRTIs as the amount of RT activity is decreased because reducing the amount of RT does not change the probability of selecting a normal nucleotide or a chain-terminating nucleotide for incorporation. The exception among NRTIs is azidothymidine (AZT), which, when incorporated, cannot translocate from the nucleotide-binding site on RT to the primer site because of steric hindrance by the large 3’-azido group (14, 15). In this position, the chain-terminating nucleotide can be excised by RT by forming a dinucleotide with ATP (14, 16). The increased sensitivity to AZT in virus with decreased PR activity, first seen with PR fitness mutants (12) and then with phenotypic mixing with an active site mutant (13), shows that the RT heterodimer that incorporates AZT is not necessarily the same one that excises it.

**Assembly and Processing**

As depicted in Fig. 1B, a small number of Gag molecules traffic dimers of the RNA genome to the plasma membrane (1, 17). Once at the membrane, additional Gag proteins are recruited through Gag-Gag interactions and nonspecific Gag-RNA interactions, utilizing Gag molecules from both the cytosol and those already attached to the membrane (17–19). Gag-Pro-Pol is recruited to sites of assembly simultaneously. Although Gag oligomerization initiates budding, the process is facilitated and completed by the ESCRT family of proteins (1, 4). Each immature virion will contain ~2400 Gag monomers (4) and ~120 Gag-Pro-Pol molecules (5). For the emerging virus particle to become infectious, the HIV-1 PR must catalyze a series of cleavage events that trigger structural and morphological changes that result in the condensation of the NC-RNA core, the formation of the CA shell, and the release of viral enzymes from their precursors (Fig. 1C). For a thorough discussion of the architecture of the HIV-1 viral core, we direct you to a number of recent publications (see Ref. 20).

The HIV-1 PR is an aspartic proteinase and functions as a homodimer (Fig. 2A) (21). Each monomer contributes an aspartic acid residue to coordinate a water molecule during the proteolysis reaction. Most aspartic proteases exist as pseudodimers in eukaryotes, but the retroviral PR originates as a
monomer within the Gag-Pro-Pol polyprotein. The need for a dimer to form the active site necessitates the interaction of two Gag-Pro-Pol molecules for proteolysis to begin. Relative to the excised mature PR, dimers formed between monomers still embedded in Gag-Pro-Pol are much less stable (22, 23) and exhibit poorer enzymatic activity (23). The instability results in the embedded dimers sampling a wide variety of conformations (22, 24, 25), potentially adopting the mature dimer interface only 3–5% of the time (25). Because of this low enzymatic activity, the first cleavage events performed by the embedded PR are intramolecular (26), with the primary result being the removal of the transframe region from the N terminus of the PR domain. These processing events occur at three locations: the spacer peptides SP1 and SP2 are removed from CA and NC, respectively (Fig. 1C) (31). Although processing has proven difficult to observe in the virion, mutant particles defective for cleavage at particular sites generated intermediates consistent with the proposed order of events determined in vitro (32). Furthermore, these mutants or others that alter the processing order severely disrupt infectivity (32–35), indicating that the timing and order of cleavage are important for the assembly of the mature viral core. The mechanisms governing ordered cleavage have been difficult to uncover, largely because no discernible pattern can be found among the cleavage site amino acid sequences (36). Instead of a consensus sequence, the substrate specificity demonstrated by the HIV-1 PR appears to be dependent upon a conserved shape (37). All of the peptide substrates derived from the cleavage sites within the Gag polyprotein were shown to adopt an asymmetric extended β-strand conformation when bound in the active site of the enzyme, creating a consensus volume termed the “substrate envelope” (Fig. 2B). Molecular modeling suggests that the cleavage sites share particular hydrogen bonding patterns between the peptide backbone and the PR, but the hydrogen bonds and hydrophobic interactions between substrate side chains and the PR are not conserved among the different cleavage sites. Thus, the differences in the side chain hydrogen bonding and hydrophobic interactions may contribute to the unique processing rate for each cleavage site (38).

Assembly, budding, and proteolytic processing of Gag and Gag-Pro-Pol are intricately linked events, although the relative timing of each and the importance of that timing are questions that are still being explored. Following initiation of assembly at the membrane, complete virions are observed at the surface of the cell within 5–10 min (18, 39), and virion release occurs 15–20 min later (18). It has been difficult to observe processing events in released virions, and early activation of PR activity by creating a tethered dimer within a single Gag-Pro-Pol molecule or delay of particle formation relative to processing negatively impacts particle formation (40, 41); these observations suggest that activation of the PR is delayed until later in the assembly process but that processing must be completed either during budding or relatively quickly after budding. PR activity is not required for the initiation of assembly (18), but there is some evidence that the presence of a PR with decreased activity can slow the rate of virion release (42). The excess of Gag over Gag-Pro-Pol (20:1) suggests that the vast majority of Gag-Pro-Pol will have primary interactions with Gag molecules. Thus, the infrequent juxtaposition of two Gag-Pro-Pol precursors in the budding Gag shell spatially limiting the number of Pro-Pol interactions, and the poor stability of the immature PR dimer in the Gag-Pro-Pol homodimer all reduce the likelihood of significant PR activity early in the assembly process. The slow or delayed release of the first PR dimer can then initiate cleavage events in trans that would include the release of
MINIREVIEW: HIV-1 Processing and Assembly

PR monomers that could dimerize and have high levels of activity. Still, it must be acknowledged that we do not know when processing is initiated, although most textbook conceptualizations of processing have it occurring after budding. We are likely to know the answer to this question when fluorescent proteins are incorporated into the virion that can also serve as substrates for the PR.

Protease Inhibitors, Resistance, and Viral Fitness

Due to the requirement of the maturation process to produce infectious virions, PR has been a major target for developing antiretroviral inhibitors, with nine PIs approved for clinical use. These PIs are transition state analogs, mostly peptidomimetics, that bind the enzyme with much higher affinity than do the substrates (21). The binding affinity of the PIs for the wild-type enzyme ranges from nM to pM (43) under conditions in which peptide substrates bind with affinities in the high µM range. The effectiveness of the PIs in antiretroviral therapy can be compromised by the emergence of resistance mutations in the PR region. More resistance mutations have been selected by PIs than any other antiretroviral drugs. Although mutations at as many as 46 positions of the 99 residues in PR have been shown to be associated with selection by PIs, only a subset of ~26 positions have been identified as those most commonly involved in PI resistance (see Refs. 44 and 45 and references therein). High level resistance to a PI typically requires four to six mutations, and PIs are thus considered to have a high genetic barrier (46). The ability to make tight binding transition state analogs that require multiple mutations to confer resistance suggests that it may be possible eventually to treat HIV-1 with a single PI if it were sufficiently potent. Efforts along these lines have been explored with some success (47–49).

There are several mechanistic features of resistance to PI. First, mutations in the active site can change the interaction with the inhibitor either by reducing a contact or creating a steric hindrance (Fig. 2A) (50–55). Such changes are more easily tolerated if a side chain of the drug extends outside of the substrate envelope. However, when such changes also impact interaction with the substrate, this results in a fitness cost in how well the enzyme functions in the context of replication (12, 56–58). Second, the fitness cost associated with these active site mutations can be compensated by mutations that occur outside of the active site but appear to be capable of enhancing PR activity (51, 54, 59–65). Although the active site mutations are absent in the untreated population, the compensatory mutations pre-exist in the population, perhaps compensating for deleterious mutations in PR that can get fixed fortuitously. Third, cleavage sites around SP2 can become limiting for making an infectious virus, and cleavage site sequences can undergo evolution to make them more easily cleaved by the mutant PR (66–70). Fourth, other mutations in Gag have been described that appear to contribute to PI resistance (71) but in unknown ways, suggesting that there are other pathways to at least low level resistance.

Our view is that the concept of fitness is synonymous with the idea of PR acting in a closed system. When PR loses activity on its normal Gag substrate cleavage sites, the probability of completing assembly and processing to yield an infectious particle is reduced, i.e. the virus is less fit to produce the full complement of infectious virus. Thus, in some assays, a single resistance-associated mutation will actually sensitize the virus to an inhibitor when the fitness loss in substrate recognition is greater than the fitness gain in resistance (13). This balance shifts as multiple resistance mutations and compensatory mutations are added. Such fitness cost and pleiotropic effects of a virus with reduced PR activity may be the reason that patients with virus carrying PI resistance mutations (that lower fitness overall) can have slowed disease progression in the setting of drug failure (72).

Assembly Inhibitors

HIV-1 particle assembly is a highly ordered process and involves the association and rearrangement of several thousand viral structural proteins. One key step involves cleavage at the N terminus of CA by the viral PR, followed by the formation of a new β-hairpin structure anchored by a salt bridge between the released N-terminal Pro-1 of CA and an internal aspartic acid side chain in CA (Asp-51 in HIV-1), an essential step in the proper assembly of the capsid cone (73, 74). Disrupting this salt bridge is an attractive drug target, although, as yet, an unrealized target. The fully processed CA makes key intermolecular CA-CA interactions that result in hexameric (and some pentameric) rings that are the basic structural unit of the conical capsid (75). Due to the indispensable nature of these interactions in generating infectious virus particles, there is an ongoing search for molecules that bind CA and inhibit these interactions. A 12-mer peptide (capsid assembly inhibitor (CAI)) and a small molecule (CAP-1) are able to disrupt HIV-1 CA assembly. CAI, a helical peptide selected in a phage display, binds to a hydrophobic cleft within the C-terminal domain (CTD) of CA (76, 77), and CAP-1 bind to the N-terminal domain (NTD) of CA, forming a hydrophobic pocket (78, 79). Recently, new CA inhibitors have been identified in high throughput screening assays. PF74, a small molecule, binds to the NTD of HIV-1 CA, near the CAP-1-binding site, and inhibits both early and late events of viral replication (80, 81). Two more series of inhibitors have been identified based on benzodiazepines (BDs) and benzimidazoles (BMs), which also bind to the same NTD of CA as CAP-1 (82). It has been proposed that all of these inhibitors are interfering with a critical NTD-CTD intermolecular interaction of CA-CA that stabilizes the hexameric and pentameric rings (82).

The structural changes that must occur during virion maturation represent one type of target in inhibiting assembly. Another target is the processing sites themselves. Blocking cleavage at a specific processing site is analogous to blocking viral DNA synthesis with a chain-terminating analog; in each case, the enzyme (PR or RT) is not inhibited, but rather its substrate (a cleavage site or the growing DNA chain) is blocked. Processing at each site in Gag is essential for making an infectious particle (32, 33, 83), although mutations blocking processing at the NC/SP2 site do not completely ablate infectivity (33, 84). Bevirimat, the prototype HIV-1 maturation inhibitor identified in a screen for inhibition of viral replication, specifically inhibits the cleavage event between CA and SP1 within the Gag polyprotein (85, 86). The drug is incorporated into immature
particles near the CA/SP1 cleavage site and stabilizes an immat-
ure form of the CA lattice, and this interaction with Gag alters
its ability to serve as a PR substrate at the CA/SP1 site (87, 88).
Recently, direct binding of bevirimat to the CA/SP1 cleavage
site in immature Gag particles has been reported (89). The dra-
matic effect on infectivity of bevirimat binding to the CA/SP1
site is similar to the effect of a processing mutant at this site,
especially if a fortuitous cleavage site within SP1 is absent (90).
Thus, bevirimat provides a proof of concept for an inhibitor of
a specific processing site. Along these lines, cleavage at the
MA/CA site must go to near-completion to make an infectious
particle. Virus particles with other CA assembly defects often dis-
play similar ring-shaped capsid-like structures, presumably
aberrant CA assemblies, regardless of the type of maturation
inhibitors used.

Another potential step in the life cycle that is impacted by PR
processing is the condensation of the viral dimeric RNA. In the
absence of processing, viral RNA is in a low stability dimeric
form in the virion (92). With processing, the RNA is in a much
more stable dimeric form. Condensation of the RNA is medi-
at ed by the NC region after it is released from Gag (93). During
the maturation process, NC is found within four different pro-
teins: full-length Gag, NC/SP2/p6 (p15), NC/SP2, and fully
released NC. These different versions of the NC protein may
have distinct functions at different steps in the life cycle (94),
providing a role for processing in the regulation of RNA binding
by the NC domain. Furthermore, there is evidence that nucleic
acid binding can regulate the efficiency of cleavage at the
SP2/p6 site using a p15 substrate (95, 96). Thus, on several
levels, processing around the NC domain of Gag is involved in
regulation of the protein activity.

Looking Ahead

Answering the question of when processing occurs in the
budding pathway is central to our understanding of virion mor-
phogenesis, and we are likely to know the answer to this ques-
tion with the application of new technologies. Our detailed
understanding of the role of protein processing in the regula-
tion of protein function for the proteins present in Gag is cre-
at ing opportunities to design assays amenable for use in high
throughput screens to search for lead compounds that can
inhibit the assembly of an infectious particle (97–99). The 25
years of studying the biochemistry of the HIV-1 virion was built
on an earlier 15 years of studying other retroviruses, starting
with the identification of a Gag precursor in avian myeloblas-
tosis virus (100). We are now at a point where we understand
critical steps in virion assembly at the molecular level and can
conceptualize new ways of disrupting these essential processes.

Acknowledgments—We thank Dr. Celia Schiffer and her colleagues
for many helpful discussions.

REFERENCES
1. Bieniasz, P. D. (2009) The cell biology of HIV-1 virion genesis. Cell Host Microbe 5, 550–558
2. Weiss, E. R., and Göttlinger, H. (2011) The role of cellular factors in promoting HIV budding. J. Mol. Biol. 410, 525–533
3. Sundquist, W. L. and Krausslich, H. G. (2012) HIV-1 Assembly, Budding, and Maturation. Cold Spring Harb. Perspect. Med. 2, a006924
4. Carlson, L. A., Briggs, J. A., Glass, B., Riches, J. D., Simon, M. N., Johnson, M. C., Müller, B., Grünewald, K., and Kräusslich, H. G. (2008) Three-
dimensional analysis of budding sites and released virus suggests a re-
vised model for HIV-1 morphogenesis. Cell Host Microbe 4, 592–599
5. Jacks, T. (1990) Translational suppression in gene expression in retrovi-
ruses and retrotransposons. Curr. Top. Microbiol. Immunol. 157, 93–124
6. Swanstrom, R., and Wills, J. W. (1997) in Retroviruses (Coffin, J. M., Hughes, S. H., and Varmus, H. E., ed) pp. 263–334, Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, NY
7. Briggs, J. A., Simon, M. N., Gross, I., Kräusslich, H. G., Fuller, S. D., Vogt,
MINIREVIEW: HIV-1 Processing and Assembly

V. M., and Johnson, M. C. (2004) The stoichiometry of Gag protein in HIV-1. Nat. Struct. Mol. Biol. 11, 672–675
8. Ambrose, Z., Julius, J. G., Boyer, P. L., Kewalramani, V. N., and Hughes, S. H. (2006) The level of reverse transcriptase (RT) in human immunodeficiency virus type 1 particles affects susceptibility to nonnucleoside RT inhibitors but not to lamivudine. J. Virol. 80, 2578–2581
9. Shen, L., Peterson, S., Sedaghat, A. R., McMahon, M. A., Callender, M., Zhang, H., Zhou, Y., Pitt, E., Anderson, K. S., Acosta, E. P., and Siliciano, R. F. (2008) Dose-response curve slope sets class-specific limits on inhibitor potential of anti-HIV drugs. Nat. Med. 14, 762–766
10. Sampah, M. E., Shen, L., Jilek, B. L., and Siliciano, R. F. (2011) Dose-response curve slope is a missing dimension in the analysis of HIV-1 drug resistance. Proc. Natl. Acad. Sci. U.S.A. 108, 7613–7618
11. Shen, L., Rabi, S. A., Sedaghat, A. R., Shan, L., Lai, J., Xing, S., and Siliciano, R. F. (2011) A critical subset model provides a conceptual basis for the high antiviral activity of major HIV drugs. Sci. Transl. Med. 3, 91ra63
12. de la Carrière, L. C., Paulous, S., Clavel, F., and Mammano, F. (1999) Effects of human immunodeficiency virus type 1 resistance to protease inhibitors on reverse transcriptase processing, activity, and drug sensitivity. J. Virol. 73, 3455–3459
13. Henderson, G. J., Lee, S. K., Irlbeck, D. M., Harris, J., Kline, M., Pollom, E., Parkin, N., and Swanstrom, R. (2012) Interplay between single resistance-associated mutations in the HIV-1 protease and viral infectivity, protease activity, and inhibitor sensitivity. Antimicrob. Agents Chemother. 56, 623–633
14. Bany, P. L., Sarafianos, S. G., Arnold, E., and Hughes, S. H. (2001) Selective excision of AZT TMP by drug-resistant human immunodeficiency virus reverse transcriptase. J. Virol. 75, 4832–4842
15. Tong, W., Lu, C. D., Sharma, S. K., Matsuura, S., So, A. G., and Scott, W. A. (1997) Nucleotide-induced stable complex formation by HIV-1 reverse transcriptase. Biochemistry 36, 5749–5757
16. Meyer, P. R., Matsuura, S. E., Mian, A. M., So, A. G., and Scott, W. A. (1999) A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. Mol. Cell. 4, 35–45
17. Jouvenet, N., Simon, S. M., and Bieniasz, P. D. (2009) Imaging the interaction of HIV-1 genomes and Gag during assembly of individual viral particles. Proc. Natl. Acad. Sci. U.S.A. 106, 19114–19119
18. Ivanchenko, S., Godinez, W. J., Lampe, M., Kräusslich, H. G., Eils, R., Rohr, K., Bräuchle, C., Müller, B., and Lamb, D. C. (2009) Dynamics of HIV-1 assembly and release. PLoS Pathog. 5, e1000652
19. Kutluay, S. B., and Bieniasz, P. D. (2010) Analysis of the initiating events in HIV-1 particle assembly and genome packaging. PLoS Pathog. 6, e1001200
20. Ganser-Pornillos, B. K., Yeager, M., and Pornillos, O. (2012) Assembly and architecture of HIV. Adv. Exp. Med. Biol. 726, 441–465
21. Lefebvre, E., and Schiffer, C. A. (2008) Resilience to resistance of HIV-1 protease inhibitors: profile of darunavir. AIDS Rev. 10, 131–142
22. Agniswamy, J., Sayer, J. M., Weber, I. T., and Louis, J. M. (2012) Terminal interface conformations modulate dimer stability prior to amino-termi

28. Pettit, S. C., Everitt, L. E., Choudhury, S., Dunn, B. M., and Kaplan, A. H. (2004) Initial cleavage of the human immunodeficiency virus type 1 Gag-Pol precursor by its activated protease occurs by an intramolecular mechanism. J. Virol. 78, 8477–8485
29. Ericsson-Viitanen, S., Manfredi, J., Viitanen, P., Tribe, D. E., Tritt, R., Hutchinson, C. A., 3rd, Loeb, D. D., and Swanstrom, R. (1998) Cleavage of HIV-1 Gag polyprotein synthesized in vitro: sequential cleavage by the viral protease. AIDS Res. Hum. Retroviruses 5, 577–591
30. Pettit, S. C., Lindquist, J. N., Kaplan, A. H., and Swanstrom, R. (2005) Processing sites in the human immunodeficiency virus type 1 (HIV-1) Gag-Pro-Pol precursor are cleaved by the viral protease at different rates. Retrovirology 2, 66
31. Pettit, S. C., Moody, M. D., Wehbie, R. S., Kaplan, A. H., Nantermet, P. V., Klein, C. A., and Swanstrom, R. (1994) The P2 domain of human immunodeficiency virus type 1 Gag regulates sequential proteolytic processing and is required to produce fully infectious virions. J. Virol. 68, 8017–8027
32. Wiegers, K., Rutter, G., Kottler, H., Tessmer, U., Hohenberg, H., and Kräusslich, H. G. (1998) Sequential steps in human immunodeficiency virus particle maturation revealed by alterations of individual Gag polyprotein cleavage sites. J. Virol. 72, 2846–2854
33. Lee, S. K., Harris, J., and Swanstrom, R. (2009) A strongly transdominant mutation in the human immunodeficiency virus type 1 gag gene defines an Achilles heel in the virus life cycle. J. Virol. 83, 8536–8543
34. Müller, B., Anders, M., Akiyama, H., Welsch, S., Glass, B., Nikovics, K., Clavel, F., Tervo, H. M., Keplert, O. T., and Kräusslich, H. G. (2009) HIV-1 Gag processing intermediates trans-dominantly interfere with HIV-1 infectivity. J. Biol. Chem. 284, 29692–29703
35. Tritt, R. J., Cheng, Y. E., Yin, F. H., and Ericsson-Viitanen, S. (1991) Mutagenesis of protease cleavage sites in the human immunodeficiency virus type 1 Gag polyprotein. J. Virol. 65, 922–930
36. Pettit, S. C., Simsic, J., Loeb, D. D., Everitt, L., Hutchinson, C. A., 3rd, and Swanstrom, R. (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
37. Prabu-Jeyabalan, M., Nalivaika, E., and Schiffer, C. A. (2002) Substrate shape determines specificity of recognition for HIV-1 protease: analysis of crystal structures of six substrate complexes. Structure 10, 369–381
38. Ozen, A., Halliköögla, T., and Schiffer, C. A. (2011) Dynamics of preferrential substrate recognition in HIV-1 protease: redefining the substrate envelope. J. Mol. Biol. 410, 726–744
39. Jouvenet, N., Bieniasz, P. D., and Simon, S. M. (2008) Imaging the biogenesis of individual HIV-1 virions in live cells. Nature 454, 236–240
40. Kräusslich, H. G. (1991) Human immunodeficiency virus protease dimer as component of the viral polyprotein prevents particle assembly and viral infectivity. Proc. Natl. Acad. Sci. U.S.A. 88, 3213–3217
41. Ott, D. E., Coren, L. V., and Shatzer, T. (2009) The nucleocapsid region of human immunodeficiency virus type 1 Gag assists in the coordination of assembly and Gag processing: role for RNA-Gag binding in the early stages of assembly. J. Virol. 83, 7718–7727
42. Kaplan, A. H., Manchester, M., and Swanstrom, R. (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
43. Anderson, J., Schiffer, C., Lee, S. K., and Swanstrom, R. (2009) Viral protease inhibitors. Handb. Exp. Pharmacol. 189, 85–110
44. Hoffman, N. G., Schiffer, C. A., and Swanstrom, R. (2003) Covariation of amino acid positions in HIV-1 protease. Virology 314, 536–548
45. Rhee, S. Y., Taylor, J., Fessel, W. J., Kaufman, D., Towner, W., Troia, P., Ruane, P., Hellinger, J., Shirvani, V., Zolopa, A., and Shafer, R. W. (2010) HIV-1 protease mutations and protease inhibitor cross-resistance. Antimicrob. Agents Chemother. 54, 4253–4261
46. Watkins, T., Resch, W., Irlebeck, D., and Swanstrom, R. (2003) Selection of high-level resistance to human immunodeficiency virus type 1 protease inhibitors. Antimicrob. Agents Chemother. 47, 759–769
47. Arribas, J. R., Pulido, F., Delgado, R., Lorenzo, A., Miralles, P., Arranz, A., Gonzalez-García, J. J., Cepeda, C., Hervás, R., Paño, J. R., Gayà, F., Carcas, A., Montes, M. L., Costa, J. R., and Peña, J. M. (2005) Lopinavir/ritonavir
as single-drug therapy for maintenance of HIV-1 viral suppression: 48-week results of a randomized, controlled, open-label, proof-of-concept pilot clinical trial (OK Study). J. Acquir. Immune Defic. Syndr. 40, 280–287

48. Katlama, C., Valantin, M. A., Algarte-Genin, M., Duvierez, C., Lambert-Niclot, S., Girard, P. M., Molina, J. M., Hoen, B., Pakianather, S., Peytavin, G., Marcelin, A. G., and Flandre, P. (2010) Efficacy of darunavir/ritonavir maintenance monotherapy in patients with HIV-1 viral suppression: a randomized open-label, noninferiority trial, MONO-ANRS 136. AIDS 24, 2365–2374

49. Valantin, M. A., Lambert-Niclot, S., Flandre, P., Morand-Joubert, L., Cabié, A., Meynard, J. L., Ponscarme, D., Ajana, F., Slama, L., Curjol, A., Cuzin, L., Schneider, L., Taburet, A. M., Marcelin, A. G., and Katlama, C. (2012) Long-term efficacy of darunavir/ritonavir monotherapy in patients with HIV-1 viral suppression: week 96 results from the MONO-ANRS 136 study. J. Antimicrob. Chemother. 67, 691–695

50. Gulnik, S. V., Suvorov, L. I., Liu, B., Yu, B., Anderson, B., Mitsuha, H., and Erickson, J. W. (1995) Kinetic characterization and cross-resistance patterns of HIV-1 protease mutants selected under drug pressure. Biochemistry 34, 9282–9287

51. Nijhuis, M., Schuurman, R., de Jong, D., Erickson, J., Gustchina, E., Albert, J., Schipper, P., Gulnik, S., and Boucher, C. A. (1999) Increased fitness of drug-resistant HIV-1 protease as a result of acquisition of compensatory mutations during suboptimal therapy. AIDS 13, 2349–2359

52. Partaledis, J. A., Yamaguchi, K., Tisdale, M., Blair, E. E., Falcione, C., Valantin, M. A., Lambert-Niclot, S., Flandre, P., Morand-Joubert, L., and Cuzin, L. (1994) Selection of multiple human immunodeficiency virus type 1 drug-resistant mutations in Gag cleavage sites. J. Biol. Chem. 269, 2806–2810

53. Bally, F., Martinez-Picado, J., Savara, A. V., Sutton, L., and D’Aquila, R. T. (1999) Replicative fitness of protease inhibitor-resistant mutants of human immunodeficiency virus type 1. J. Virol. 73, 3744–3752

54. Koch, N., Yahi, N., Fantini, J., and Tamalet, C. (2001) Mutations in HIV-1 protease confer reduced fitness and drug susceptibility in vitro. J. Biol. Chem. 276, 3265–3268

55. Zennou, V., Mammano, F., Petit, C., and Clavel, F. (1998) Resistance-associated loss of viral fitness in human immunodeficiency virus type 1: phenotype analysis of protease and Gag coevolution in protease inhibitor-treated patients. J. Virol. 72, 7632–7637

56. Zhang, Y. M., Imamichi, H., Imamichi, T., Lane, H. C., Falloon, J., Albertine, K. H., Schipper, P., Gulnik, S., and Boucher, C. A. (1999) Increased fitness of drug-resistant HIV-1 protease as a result of acquisition of compensatory mutations selected under drug pressure. J. Biol. Chem. 274, 3265–3268

57. Doyon, L., Croteau, G., Thibeault, D., Poilvin, F., Pilotte, L., and Lamarré, D. (1996) Selection of multiple human immunodeficiency virus type 1 resistance to protease inhibitors. J. Virol. 70, 3763–3769

58. Parry, C. M., Kohli, A., Boinett, C. J., Towers, G. J., McCormick, A. L., and Pillay, D. (2009) Gag determinants of fitness and drug susceptibility in protease inhibitor-resistant human immunodeficiency virus type 1. J. Virol. 83, 9094–9101

59. Deeks, S. G., Hoh, R., Grant, R. M., Winr, T., Carbone, J. D., Narváez, A., Cesar, D., Abe, K., Hanley, M. B., Hellmann, N. S., Petropoulos, C. J., McCune, J. M., and Hellerstein, M. K. (2002) CD4+ T cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy. J. Infect. Dis. 185, 315–323

60. Mortuza, G. B., Haire, L. F., Stevens, A., Smerdon, S. J., Stoye, J. P., and Taylor, I. A. (2004) High-resolution structure of a retroviral capsid hexameric amino-terminal domain. Nature 431, 481–485

61. von Schwedler, U. K., Stemmler, T. L., Klishko, V. Y., Li, S., Albertine, K. H., Davis, D. R., and Sundquist, W. I. (1998) Proteolytic refolding of the HIV-1 capsid protein amino terminus facilitates viral core assembly. EMBO J. 17, 1555–1568

62. Kelly, B. N., Kyere, S., Kinde, I., Tang, C., Howard, B. R., Robinson, H., Sundquist, W. I., Summers, M. F., and Hill, C. P. (2007) Structure of the antiviral assembly inhibitor CAP-1 complex with the HIV-1 CA protein. J. Mol. Biol. 373, 355–366

63. Tang, C., Loeliger, E., Kinde, I., Kyere, S., Mayo, K., Barklis, E., Sun, Y., Huang, M., and Summers, M. F. (2003) Antiviral inhibition of the HIV-1 capsid protein. J. Mol. Biol. 327, 1013–1020

64. Blair, W. S., Pickford, C., Irving, S. L., Brown, D. G., Anderson, M., Bazin, R., Cao, J., Ciaramella, G., Isacson, J., Jackson, L., Hunt, R., Kjerström,
MINIREVIEW: HIV-1 Processing and Assembly

A., Nieman, J. A., Patick, A. K., Perros, M., Scott, A. D., Whitby, K., Wu, H., and Butler, S. L. (2010) HIV capsid is a tractable target for small molecule therapeutic intervention. *PloS Pathog* 6, e1001220

81. Shi, J., Zhou, J., Shah, V. B., Aiken, C., and Whitby, K. (2011) Small-molecule inhibition of human immunodeficiency virus type 1 infection by virus capsid destabilization. *J. Virol.* 85, 542–549

82. Lemke, C. T., Titolo, S., von Schwedler, U., Goudreau, N., Mercier, J. F., Wardrop, E., Faucher, A. M., Coulombe, R., Banik, S. S., Fader, L., Gagnon, A., Kawai, S. H., Rancourt, J., Tremblay, M., Yoakim, C., Simonneau, B., Archambault, J., Sundquist, W. L., and Mason, S. W. (2012) Distinct effects of two HIV-1 capsid assembly inhibitor families that bind the same site within the N-terminal domain of the viral CA protein. *J. Virol.* 86, 6643–6655

83. Pettit, S. C., Henderson, G. J., Schiffer, C. A., and Swanstrom, R. (2002) Replacement of the P1 amino acid of human immunodeficiency virus type 1 Gag processing sites can inhibit or enhance the rate of cleavage by the viral protease. *J. Virol.* 76, 10226–10233

84. Coren, L. V., Thomas, J. A., Chertova, E., Sowder, R. C., 2nd, Gagliardi, A. C. (2011) HIV-1 maturation inhibitor bevirimat stabilizes the immature Gag lattice. *J. Virol.* 85, 6207–6214

85. Pettit, S. C., Tritch, R. J., and Ott, D. E. (2007) Mutational analysis of the C-terminal Gag cleavagesites in human immunodeficiency virus type 1. *J. Virol.* 81, 10047–10054

86. Li, F., Goila-Gaur, R., Salzwedel, K., Kilgore, N. R., Reddick, M., Matalana, C., Castillo, A., Zoumplis, D., Martin, D. E., Orenstein, J. M., Allaway, G. P., Freed, E. O., and Wild, C. T. (2003) PA-457: a potent HIV protease-defective virions. *J. Virol.* 77, 347–63

87. Rulli, S. J., Jr., Muriaux, D., Nagashima, K., Mirro, J., Oshima, M., Baumann, J. G., and Rein, A. (2006) Mutant murine leukemia virus Gag proteins lacking proline at the N terminus of the capsid domain block infectivity in virions containing wild-type Gag. *Virology* 347, 364–371

88. Fu, W., Gorelick, R. J., and Rein, A. (1994) Characterization of human immunodeficiency virus type 1 dimeric RNA from wild-type and protease-defective virions. *J. Virol.* 68, 5013–5018

89. Shehu-Xhilaga, M., Krausslich, H. G., Pettit, S., Swanstrom, R., Lee, J. Y., Marshall, J. A., Crowe, S. M., and Mak, J. (2001) Proteolytic processing of the p2/nucleocapsid cleavage site is critical for human immunodeficiency virus type 1 RNA dimer maturation. *J. Virol.* 75, 9156–9164

90. Thomas, J. A., and Gorelick, R. J. (2008) Nucleocapsid protein function in early infection processes. *Virus Res.* 134, 39 – 63

91. King, N. M., Prabu-Jeyabalan, M., Nalivaika, E. A., and Schiffer, C. (2004) Cleavage of p15 protein in vitro by human immunodeficiency virus type 1 protease is RNA-dependent. *J. Virol.* 68, 6207–6214

92. Sheng, N., Pettit, S. C., Tritch, R. J., Ozturk, D. H., Rayner, M. M., Swanstrom, R., and Erickson-Viitanen, S. (1997) Determinants of the human immunodeficiency virus type 1 p15NC-RNA interaction that affect enhanced cleavage by the viral protease. *J. Virol.* 71, 5723–5732

93. Breuer, S., Chang, M. W., Yuan, J., and Torbett, B. E. (2012) Identification of HIV-1 inhibitors targeting the nucleocapsid protein. *J. Med. Chem.* 55, 4968–4977

94. Breuer, S., Sepulveda, H., Chen, Y., Trotter, J., and Torbett, B. E. (2011) A cleavage enzyme-cytometric bead array provides biochemical profiling of resistance mutations in HIV-1 Gag and protease. *Biochemistry* 50, 4371–4381

95. Herrme, J., Anders, M., Heuser, A. M., and Müller, B. (2010) A simple fluorescence based assay for quantification of human immunodeficiency virus particle release. *BMC Biotechnol.* 10, 32

96. Vogt, V. M., and Eisenman, R. (1973) Identification of a large polypeptide precursor of avian oncornavirus proteins. *Proc. Natl. Acad. Sci. U.S.A.* 70, 1734–1738

97. King, N. M., Prabu-Jeyabalan, M., Nalivaika, E. A., and Schiffer, C. (2004) Combating susceptibility to drug resistance: lessons from HIV-1 protease. *Chem. Biol.* 11, 1333–1338