

# The Packaging and Maturation of the HIV-1 Pol Proteins

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**Abstract:** The Pol protein of human immunodeficiency virus type 1 (HIV-1) harbours the viral enzymes critical for viral replication; protease (PR), reverse transcriptase (RT), and integrase (IN). PR, RT and IN are not functional in their monomeric forms and must come together as either dimers (PR), heterodimers (RT) or tetramers (IN) to be catalytically active. Our knowledge of the tertiary structures of the functional enzymes is well advanced, and substantial progress has recently been made towards understanding the precise steps leading from Pol protein synthesis through viral assembly to the release of active viral enzymes. This review will summarise our current understanding of how the Pol proteins, which are initially expressed as a Gag-Pol fusion product, are packaged into the assembling virion and discuss the maturation process that results in the release of the viral enzymes in their active forms. Our discussion will focus on the relationship between structure and function for each of the viral enzymes. This review will also provide an overview of the current status of inhibitors against the HIV-1 Pol proteins. Effective inhibitors of PR and RT are well established and we will discuss the next generation inhibitors of these enzymes as well recent investigations that have highlighted the potential of IN and RNase H as antiretroviral targets.

**Keywords:** Gag-Pol, HIV-1, protease, reverse transcriptase, integrase.

## INTRODUCTION

The human immunodeficiency virus type 1 (HIV-1) genome has three main coding regions; *gag* which directs the synthesis of the internal structural proteins, *pol* which encodes the viral enzymes and *env* which gives rise to the envelope proteins. The *pol* gene, which lacks an initiation codon, partially overlaps and is in the -1 reading frame with respect to *gag*. As a result Pol is only synthesized as part of a Gag-Pol fusion protein. Gag is expressed as a 55 kDa precursor (Pr55-Gag) and is cleaved to produce the mature proteins; matrix (MA), capsid (CA), p2, nucleocapsid (NC), p1 and p6<sup>Gag</sup> (Fig. 1). Pol is expressed in the form of a 160 kDa Gag-Pol fusion protein (Pr160-Gag-Pol) and is cleaved to produce MA, CA, p2, NC, the transframe protein (TF), and the viral enzymes protease (PR), reverse transcriptase (RT) and integrase (IN) (Fig. 1). The viral enzymes catalyse a number of essential steps in HIV-1 replication (summarized in Fig. 2). PR catalyses the proteolytic processing of the Gag and Gag-Pol precursor proteins. RT catalyses the conversion of single stranded RNA into double stranded viral DNA for integration into the host chromosomes. RT is a multifunctional enzyme with RNA-directed DNA polymerase, DNA directed DNA polymerase and ribonuclease hybrid activities (RNase H). IN mediates the integration of the linear double stranded viral DNA into the host cell chromosome. The multimerization of the polymerase subunits is critical for their enzymatic function

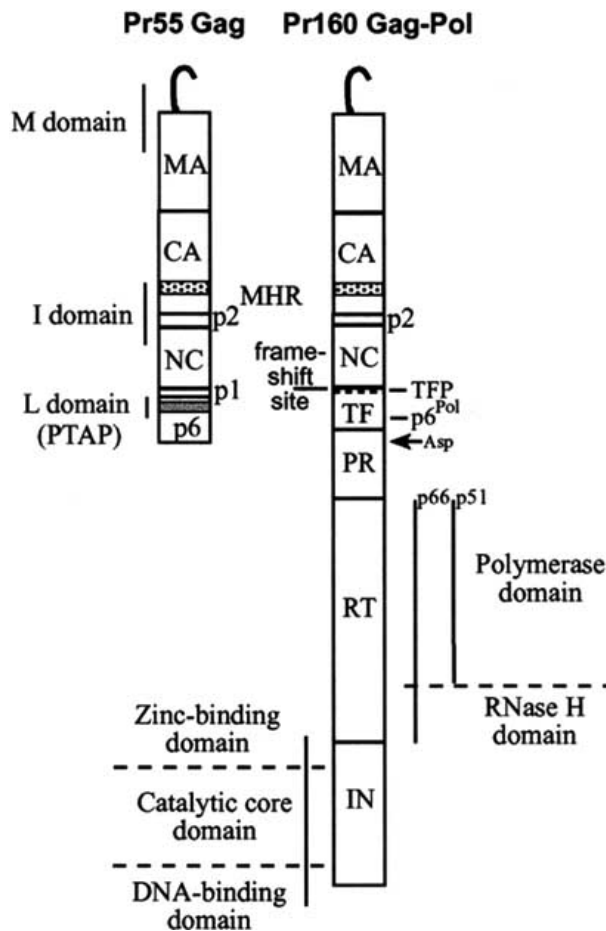
and PR, RT and IN exist as either dimers (PR), heterodimers (RT) or tetramers (IN). This review will follow the path the viral enzymes take through Gag-Pol protein synthesis, packaging into the assembling virion and the maturation process that delivers active PR, RT and IN.

## EXPRESSION OF THE GAG-POL POLYPROTEIN

HIV-1, like other viruses, makes the most of its compact genome by using novel modes of gene expression to allow the synthesis of multiple proteins from a single RNA species. In all retroviruses the structural proteins (encoded by the *gag* gene) and the enzymatic proteins (encoded by the *pol* gene) are synthesised from a single RNA template as polyproteins with a common N-terminus. Thus, translation of the viral genome results in the formation of both a Gag precursor and a Gag-Pol precursor that is produced at 10-20 fold lower level than Gag (for review see [103]). In the case of gammaretroviruses such as the feline leukemia virus (FeLV) and the murine leukemia virus (MLV), *gag* and *pol* are in the same reading frame and the Pol protein is expressed as the result of an infrequent translational readthrough of the *gag* termination codon [66]. In contrast, in lentiviruses such as HIV-1 and the alpharetrovirus avian leukosis virus (ALV), *pol* is in a different reading frame to *gag* and a small proportion of ribosomes undergo a -1 ribosomal frameshifting event to facilitate Pol protein expression [77, 78, 147, 186].

In HIV-1 programmed -1 ribosomal frameshifting occurs at a level of 5-10% of Gag synthesis [79], ensuring maintenance of a strict ratio of 20 Gag molecules to each Gag-Pol. This 20:1 ratio of Gag to Gag-Pol is critical for

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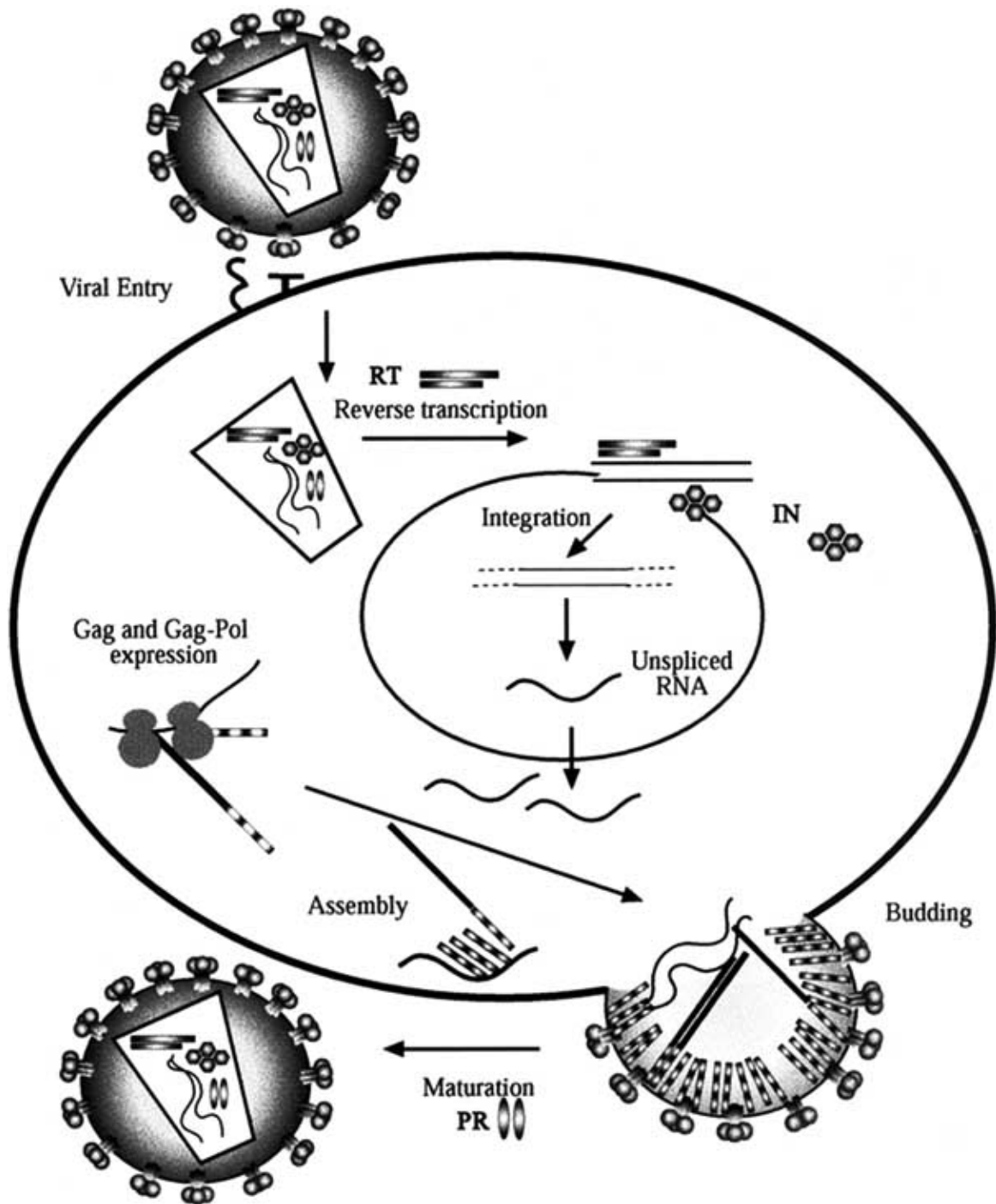
**Fig. (1).** Schematic representation of some of the major features of the Gag and Gag-Pol polyproteins.

RNA dimerization, viral assembly, replication and infectivity [88, 134, 162]. The frameshift required for the translation of HIV-1 Gag-Pol is promoted by two *cis*-acting RNA components a UUUUUUA heptamer termed the “slippery” sequence and a stem-loop (Fig. 3). These elements are located in the overlapping region of the *gag* and *pol* genes directly following the coding region for NC. Sequence analysis has shown that the UUUUUUA sequence is highly conserved [9]. Moreover, Biswass *et al.* [9] have demonstrated that any changes to the heptamer, including changes that allow frameshifting in other viruses, will decrease the efficiency of frameshifting and inhibit infectivity in HeLa cells [9]. Traditionally the frameshift stem-loop structure has been thought of as a simple 12bp stem-loop, separated from the slippery sequence by a 7 nucleotide spacer sequence [79, 85, 101] (Fig. 3A). The first direct structural information on this traditional stem-loop was recently described by Staple and Butcher [175]. Their nuclear magnetic resonance (NMR) study revealed that the frameshift stem-loop is an A-form helix capped by a structured ACAA tetraloop [175]. Two groups have now shown that the frameshift region extends beyond the simple stem-loop giving rise to a more complex signal that contributes to the frameshift mechanism [43, 45]. Dulude *et al.* [45] have suggested that the frameshift stimulatory signal consists of a two-stem helix, where the classic stem-loop has a lower stem that pairs with the spacer sequence after a 3-

purine bulge (Fig. 3B). In contrast, Dinman *et al.* [43] have proposed a triple helix structure, where the pyrimidine rich region downstream of the classic stem-loop interacts with 3 bases in the loop (Fig. 3C). Both models incorporate the stable stem-loop structure described by Staple and Butcher [175]. In addition both models were in agreement with the potential of 139 *pol* sequences in the Los Alamos National Laboratory HIV sequence database to form either a two stem helix or a triple helix [45]. Only 7.2% of sequences could not form a triple helix and only 3.6% could not form a two-stem helix. Ultimately there is a need to characterize the structure of the extended frameshift region to see which model holds true.

Although the precise mechanism of frameshifting is not completely understood, the most likely function of the secondary structure is to cause the progressing ribosomes to pause over the slippery sequence so that the ribosome-bound peptidyl-tRNA and aminoacyl-tRNA slip back from their initial position in the Gag reading frame and re-pair in the -1/Pol reading frame [80] (Fig. 3D). While the slippery sequence alone mediates a low level of frameshifting, the addition of the stem-loop increases ribosomal frameshifting efficiency 3-5 fold [7, 20, 21, 136, 149]. Stem-loop thermodynamic stability is critical for maintaining the Gag to Gag-Pol ratio. Major alterations to stem-loop stability decrease frameshifting efficiency [6, 7] and abrogate viral infectivity [70, 182]. It is also thought that a direct interaction between the stem-loop and the ribosomes may be required during translation to facilitate the -1 frameshift [85, 165, 175], in which case stem-loop stability would be critical in providing an accessible binding surface for the progressing ribosomes. Chang *et al.* [23] identified a large amount of natural variation in the stability of the frameshift stem-loop amongst 76 *gag* nucleotide sequences from various strains and subtypes of HIV-1. The average estimated thermodynamic stability ( $\Delta G^\circ$ ) of the frameshift stem-loops from 29 subtype B viruses was -21.3 kcal/mol, while that of nine subtype A stem-loops was -17 kcal/mol and five of seven subtype F sequences had an estimated stability of greater than or equal to -15 kcal/mol [23]. When assessing frameshifting efficiency across the different subtypes of Group M, Baril *et al.* [6] found little variability despite differences in stem-loop stability. In addition, Telenti *et al.* [182] reported that natural variations in thermodynamic stability that result in decreased frameshifting (up to 40%) and impaired Gag-Pol maturation were still able to support wild-type replication in PBMCs [182]. Overall it appears that rather than evolving to a strictly defined stem-loop structure, there is strong selective pressure to maintain a frameshift signal that supports the correct ratio of Gag to Gag-Pol synthesis and thereby preserve the balance between structural proteins and enzymes that is required for the production of infectious virions.

Like -1 frameshifting, translational readthrough requires sequence structures in the retroviral RNA for Pol protein expression. These include several nucleotides following the UAG and a pseudoknot ([2] and references therein). However, it was recently demonstrated that optimal translational readthrough in MuLV also requires the involvement of RT and the cell protein erf1 (eukaryotic translation release factor 1) [62, 130]. Erf1 was identified as a binding partner of RT using the yeast two-hybrid system



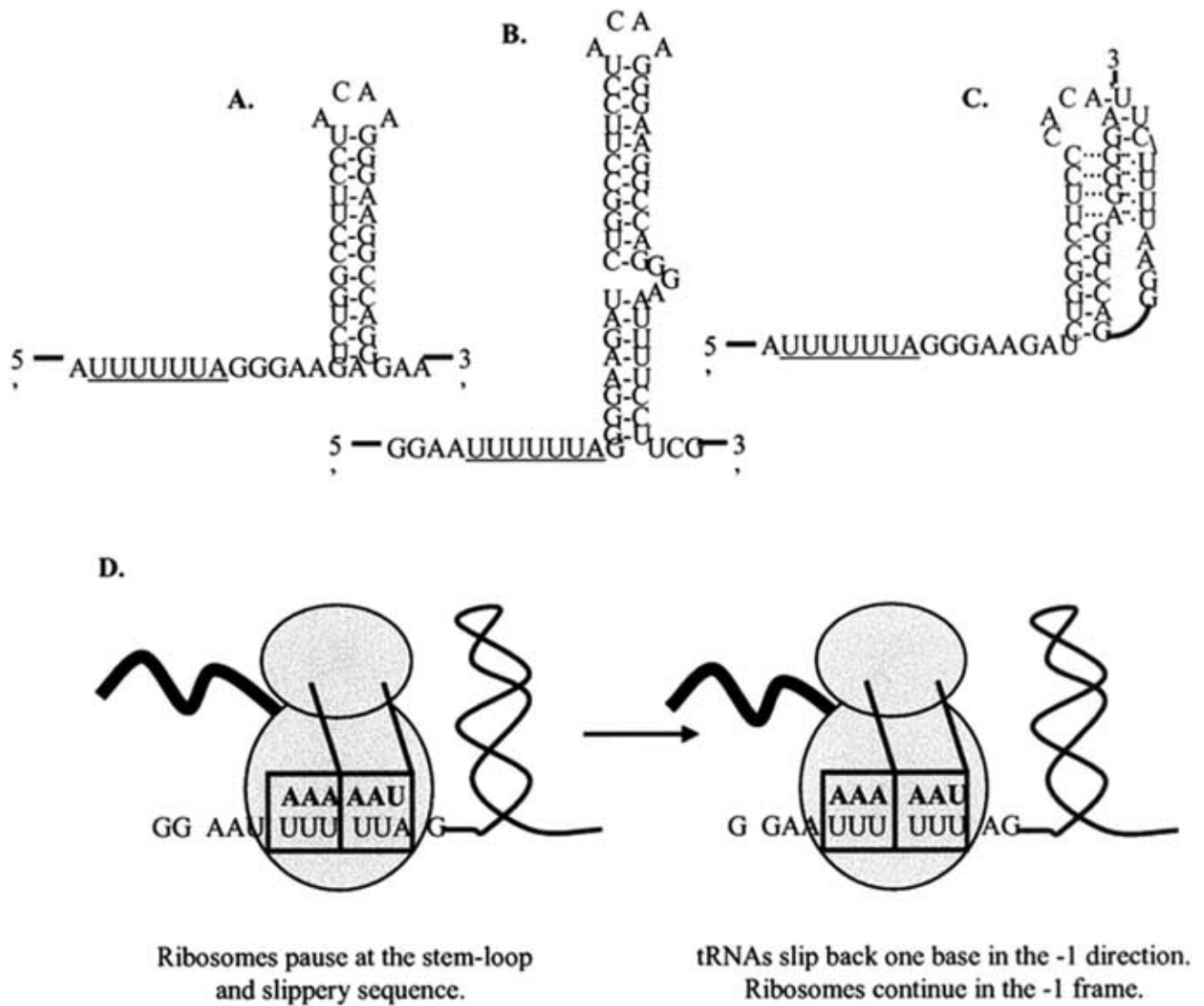
**Fig. (2).** General features of the HIV-1 replication cycle, highlighting those steps that involve the Pol enzymes, PR, RT and IN. Following receptor binding and viral entry RT catalyses the conversion of single stranded viral genomic RNA into the double stranded proviral DNA precursor for IN mediated integration into the host chromosome. The Gag and Gag-Pol proteins are synthesized in the cytoplasm as polyprotein precursors that are transported to the membrane for assembly and budding. The resultant virions are immature and non-infectious. Viral maturation is mediated by PR cleavage of the precursor polyproteins.

and it was subsequently shown using RT mutants that the interaction between RT and *erf1* is critical for Gag-Pol protein expression [130]. The ability of HIV-1 and other viruses to recruit host cell factors to support replication is notorious and although there is no current evidence, it remains possible that factors beyond the slippery sequence and the stem-loop are required for -1 frameshifting in HIV-1.

**PACKAGING THE GAG-POL PRECURSOR PROTEIN INTO THE ASSEMBLING VIRION**

**Viral Assembly**

Assembly pathways differ between cell types. While T-cells primarily support viral particle release at the plasma membrane, the virus assembles in intracellular vesicles in



**Fig. (3).** The frameshift region of HIV-1. (A) The simple stem-loop proposed by Jacks *et al.* [79] and recently investigated by Staple and Butcher [175], showing the slippery sequence (underlined) and the predicted stem-loop structure. (B) The two stem helix structure proposed by Dulude *et al.* [45]. (C) The intramolecular triplex structure proposed by Dinman *et al.* [43]. (D) A model for the mechanism of ribosomal frameshifting.

macrophages. This review will cover assembly in general terms, focusing on particle assembly as it is currently thought to occur in T-cells. Gag is the driving force behind viral assembly, assisted by host factors and cellular machinery (for review see [120]). Three key assembly domains have been described in Gag (Fig. 1). The membrane interaction (M) domain is essential for transport to the cell membrane and requires Gag to undergo post-translational myristoylation to the N-terminus of MA [60]. Gag attaches to the membrane via the myristic acid moiety and via ionic interactions between membrane phospholipids and basic amino acids within MA [203]. The interaction (I) domain is located at the N-terminus of NC [157] and is important for Gag-Gag complex formation, as recently highlighted using FRET (fluorescence resonance energy transfer) assays with wild-type and truncated Gag molecules that demonstrated the I domain of Gag mediates Gag complex formation in living cells. [40]. p6<sup>Gag</sup> contains an N-terminal PTAP motif

termed the late (L) domain which is critical for viral budding [63, 199, 200]. PTAP has been shown to interact with TSG101 and this interaction is essential for the final stages of particle budding [59, 117, 185] (for review see [54]).

Our understanding of the many facets of viral assembly continues to expand. Retroviral infection of non-dividing cells is dependent on their ability to enter the nucleus of the host cell. Following viral entry a pre-integration complex (PIC) consisting of both viral and cellular components is formed [11, 12, 13]. The viral proteins IN, Vpr and MA have been identified in the PIC and shown to have nuclear localization signals (NLSs) [11, 39, 53, 57, 68, 144]. MA has also been reported to encode a nuclear export signal [46]. Wherein, nuclear export of Gag is mediated by the Crmp1p protein export pathway [46]. It appears that newly synthesised Gag is required to enter the nucleus to facilitate the proper assembly of HIV-1 [46]. The nuclear export signal

is thought to counteract the nuclear import signal to ensure that the Gag precursor is localised to the cell cytoplasm during the process of viral assembly. However, the precise mechanisms of both nuclear entry and export have not been fully elucidated and this feature of HIV-1 assembly remains controversial as the literature contains a number of conflicting reports. For example, one study by Reil *et al.* [148] indicates that viruses deficient in MA can infect non-dividing cells at levels similar to wild-type. Interestingly, in Rous sarcoma virus (RSV) Gag has been shown to enter the nucleus via a NLS in MA and be exported using a CRM1-mediated pathway [159]. It is not yet clear whether there are any mechanistic parallels between nuclear entry and export in RSV and HIV-1 [159].

### Requirements for Gag-Pol Packaging

It is generally believed that the Gag-Pol protein, which cannot form a virus particle alone [118], is incorporated into assembling particles through interactions within the CA domains of Gag and Gag-Pol [72, 174, 193]. Efficient incorporation of Gag-Pol requires the major homology region (MHR) in CA and the adjacent C-terminal CA sequences [30, 31, 72]. Intriguingly, mutations in the CA-MHR that inhibit the packaging of Gag-Pol allow the formation of Gag particles, so it is not strictly the same sequences involved in Gag-Gag interactions that facilitate Gag/Gag-Pol interactions [72, 174].

While interactions between the CA regions of Gag and Gag-Pol are the key route for packaging the Gag-Pol precursor, Pol proteins can in fact be packaged when expressed outside the context of a Gag-Pol fusion. For example, functional RT and IN can be incorporated into VLPs when fused to Vpr [196, 197], presumably reliant on Vpr-Gag interactions [139]. Remarkably, Cen *et al.* [22] have recently demonstrated that the Pol proteins could be packaged into an assembling virion without the Gag portion of Gag-Pol. The packaging of Pol proteins occurred at 70% the level of wild-type. Moloney murine leukemia virus (Mo-MLV) Pol proteins can also be packaged when not expressed in the context of a Gag-Pol fusion protein, as shown via co-expression studies of Mo-MLV Gag and Mo-MLV Pol encoded on separate plasmids [10]. However, a decrease in RT activity was observed in the resulting virions, suggesting a deficient packaging of Pol [10]. Both studies suggest there is a direct interaction *in trans* between Pol and Gag that can facilitate the incorporation of Gag-Pol. Pol sequences important for Gag-Pol packaging are thought to lie within the N-terminus of RT as incorporation of Gag-Pol does not require RNase H or IN [92] and PR and IN were shown to be dispensable for packaging truncated Pol constructs into Gag-VLPs [22]. Cen *et al.* [22] have also suggested a role for p6<sup>Gag</sup> in the packaging of Pol proteins as deletion of p6<sup>Gag</sup> decreased Pol incorporation into Gag-VLPs 4-5 fold. Further work is required to establish whether this interaction occurs *in vivo* in the absence of the high levels of expression obtained in the described transfection system [22]. At what stage this interaction might come into play also needs to be resolved. One possibility is that p6<sup>Gag</sup> is important for retaining the Pol proteins within the virion once Gag-Pol is cleaved [22]. This suggestion is consistent with the finding that proline residues in p6<sup>Gag</sup> are important

for retaining Pol proteins following PR activation in a cell-type specific interaction [41, 199].

### Trafficking Gag-Pol from the Ribosomes to the Site of Virus Assembly

Gag and Gag-Pol are synthesized on ribosomes in the cell cytoplasm and then transported to the plasma membrane where the particle obtains its lipid envelope and buds from the host cell. Gag is thought to be responsible for transporting Gag-Pol to the cell membrane. The clearest evidence comes from studies that have shown non-myristoylated Gag and non-myristoylated Gag-Pol could be packaged into the virion if co-expressed with myristoylated Gag [28, 135, 172]. Gag contains several binding interfaces that promote Gag-Gag interactions and the model of virus assembly that is now emerging from analysis of Gag virus like particles (VLPs) strongly suggests that Gag polyproteins form small multimers in the cytoplasm and assemble into larger complexes at the cell membrane. Building on evidence from several groups that have observed Gag multimers within the cell cytoplasm [102, 106, 126] multimerized Gag complexes have now been identified at the plasma membrane and in the cytoplasm of living cells for both RSV [99] and HIV-1 [40] using FRET analysis. In addition, NMR studies have shown that the membrane targeting step of MA myristoylation is regulated by an entropic shift in a pre-existing equilibrium wherein myristate (myr) exposure is coupled with Gag self assembly [181]. These studies suggest that multimerization of Gag generates an intermediate complex in the cytoplasm that exposes the myristoylation signal and consequently facilitates transport to the plasma membrane [181]. This demonstration provides a strong argument against the possibility that Gag complexes identified in the cytoplasm represent misfolded or misdirected complexes targeted for degradation.

Although the bulk of the assembly studies have been conducted with Gag VLPs, there is also evidence that supports the idea that Gag-Pol multimerizes with Gag in the cell cytoplasm. Lee *et al.* [102] have reported the formation of Gag and Gag-Pol precursor complexes in the cytoplasm of HIV-1 infected CD4<sup>+</sup> T-cells, and more recently a direct interaction between Gag and Gag-Pol was shown with the immunoprecipitation of a Gag/Gag-Pol complex from transfected COS7 cells [65]. In the Halwani *et al.* [65] study, all newly synthesized Gag-Pol was found to be associated with Gag at the detergent-resistant membrane (DRM). While Gag is responsible for Gag-Pol packaging, the Gag-Pol precursor in turn has recently been shown to be important for the packaging of the viral accessory protein Nef through a direct interaction between the C-terminal flexible loop of Nef and the TF protein in Gag-Pol [35]. The addition of an ER retrieval signal to the C-terminus of Nef has shown that the Nef and Gag-Pol interaction is strong enough to redirect Gag-Pol from the cytoplasm to an intermediate compartment, causing interference with Gag processing and viral infectivity [35]. The resultant perinuclear localization and interfering phenotype mimics that caused by the HIV-1<sub>2</sub> Nef protein, NefF12 [50, 129] and it has been demonstrated that Gag-Pol is misrouted by NefF12 as a result of three amino acid changes in this atypical Nef protein [35]. These findings, combined with the

identification of a role for Nef in directing budding from lipid rafts [202] suggest Nef may play a supporting role in Gag-Pol packaging. A Gag/Gag-Pol/Nef assembly complex formed in the cytoplasm would have a multitude of membrane targeting motifs, potentially allowing the complex to be more efficiently transported to the cell membrane [35].

### **The Role of Viral RNA in Gag-Pol Packaging and Virion Architecture.**

It is now well established that the assembly of HIV-1 and other retroviruses is facilitated by nucleic acids [14, 16, 17, 32, 121, 124], which in turn suggests that the viral RNA may have an important role in packaging Gag-Pol into the assembling virion. Accordingly, recent investigations have shown that the viral RNA is important for the formation of stable Gag/Gag-Pol complexes [91]. Khorchid *et al.* [91] found that mutations in Gag NC that prevent Gag-RNA binding also inhibit the formation of stable Gag/Gag-Pol complexes and treatment with RNase was able to destabilize the Gag/Gag-Pol complex. The same mutation in NC of Gag-Pol had no effect on complex formation. Overall it appears that Gag-Pol does not directly interact with RNA but the formation of Gag/Gag-Pol complexes requires RNA facilitated Gag multimerization [91]. It has been speculated that the role of the I domain in mediating Gag-Gag interactions may be to enhance Gag multimerization by acting as a nucleic acid tether [40, 157]. As multiple Gag molecules bind to the RNA via the I domain, the protein-protein interaction domains of Gag are brought together allowing Gag complex formation and, as recently demonstrated by Tang *et al.* [181], exposure of MA's myristoylation signal. As Gag-Pol does not appear to interact directly with the viral RNA [91], Gag-Pol must be incorporated into the Gag and RNA complex via protein-protein interactions, presumably between the CA domains of Gag and Gag-Pol. Although translation of Gag and Gag-Pol from the same mRNA is not critical for Gag-Pol packaging [135, 172] or for viral function [69], a likely location for the initiation of RNA, Gag and Gag-Pol interaction is at the polyribosome where the constituents of the complex are in high concentration. In this environment the Gag monomers could multimerize on the RNA scaffold in such a way that the molecular organization of the proteins yields the correct positioning for incorporation of the less frequently synthesized Gag-Pol proteins. Cell based FRET assays of assembly (as described for Gag VLPs [40, 99]) that include both Gag and Gag-Pol may shed light on this issue.

The exact role of the viral RNA in particle assembly has not been fully elucidated. As described above it has been proposed that RNA acts as a scaffold for the multimerization of Gag and Gag-Pol [16, 17, 18, 40, 157]. However, Wang *et al.* [190] have recently suggested that the main role of the viral RNA is to stabilise the viral core, preventing the collapse of the particle during processing of the Gag and Gag-Pol precursor proteins. In either scenario a structural role for RNA during viral assembly, wherein the viral RNA dictates virus architecture, may facilitate the organisation of Gag-Pol molecules within the virion. This would allow the appropriate placement of the viral enzymes and support the correct molecular arrangements for proper virion function.

The conformation of the Gag-Pol precursor is important for the correct positioning of RT in the ribonucleoprotein (RNP) initiation complex as DNA synthesis by an RT defective virus cannot be rescued by exogenously supplied RT [4]. In addition, functional RT and IN proteins can be packaged when supplied *in trans* as Vpr fusion proteins to generate infectious virions [107, 196]. However, Wu *et al.* [196] found that the levels of infection achieved when the proteins were supplied individually as Vpr-RT and Vpr-IN did not reach those of virions complemented by RT-IN supplied in fusion (Vpr-RT-IN) which in turn were not as infectious as wild-type virions. Similarly, while the independent packaging of Vpr-RT and Vpr-IN into processed Gag-VLPs does not permit RNA dimer maturation or core formation, virion packaging of Vpr-RT-IN generates viral genomic RNA in the correct conformation with wild-type-like electron dense cone shaped cores [163].

### **PROTEIN PROCESSING AND VIRAL PARTICLE MATURATION**

#### **It's All in the Timing**

PR mediated processing of the Gag and Gag-Pol precursors generates a dramatic transformation in virion morphology whereby non-infectious particles with electron lucent cores are converted to infectious particles with electron dense cone shaped cores [87, 89, 94, 155]. The exact timing of Gag and Gag-Pol processing during HIV-1 replication remains uncertain. Cell-associated precursor cleavage is well established. However, whether processing for virion formation occurs at the membrane just before or during release or strictly within the assembled virion has proved difficult to elucidate. In many ways we are no closer to answering this question than a decade ago when Kaplan *et al.* [86] examined virions 10 seconds post-release and found processing was complete, narrowing the window to show that PR cleavage is initiated during or shortly after virus release from the plasma membrane of the infected cell.

The regulation of PR function and timing is critical for viral assembly. Premature activation of PR [97], partial inhibition of its activity [87] or over-expression [88] lead to major defects in assembly and the production of non-infectious particles. Efficient viral budding appears closely linked to PR activity. A reduction in viral budding and defects in protein processing generated via mutations in PTAP of p6<sup>Gag</sup> can be rescued if these mutations are present in a PR negative construct so that processing is blocked [73]. Moreover, it has been reported that virus production is slowed when processing is decreased or blocked [86, 158] and over-expression of either PR or Gag-Pol can suppress virus budding, presumably due to premature cleavage of the Gag precursors in the cell [5, 88, 97, 134]. Not surprisingly, major assembly defects resulting from the premature processing of Gag was recently shown by an NC mutant with flawed assembly which could be rescued by blocking PR activity [131].

The importance of perfect timing in PR activation was recently highlighted by Murakami *et al.* [122] who have shown that viral fusion is affected by Gag processing, suggesting a critical role for proteolytic processing as a maturational "switch". Several groups have provided

evidence to support an interaction between the MA protein of Gag and the cytoplasmic tail of the envelope protein gp41 [34, 55, 116, 123, 198]. Murakami *et al.* [122] have extended this work showing that protease negative constructs have defects in cell-cell fusion. Consequently it appears that the interaction between Gag and gp41 holds the envelope glycoprotein in a conformation that inhibits fusion, and processing of the MA protein induces a conformational change that allows fusion to occur. Proteolytic processing may also be important for modulating the activities of NC during infection as an investigation of the role of NC in stimulating integration found that of the three NC proteins tested (NCp7, NCp9 and NCp15) the intermediate precursor NCp9 generated the highest levels of integration *in vitro* [58].

**Sequential Processing and the Autocatalytic Release of PR**

PR is responsible for its own release from the Gag-Pol precursor. PR is flanked by the TF protein at the N-terminus and by the RT domain at the C-terminus. TF consists of two domains; a conserved N-terminal octapeptide (TFP) and a 48-60 amino acid variable region (p6<sup>Pol</sup>) that are separated by a cleavage site. The precise mechanisms directing PR autocatalytic release have not been resolved. The initial cleavages leading to the release of PR are presumably carried out by the precursor associated immature PR, while later cleavages are carried out by the fully processed mature PR [115, 140, 183]. Several model systems have been used to examine the autocatalysis of PR utilising PR constructs flanked by native Gag-Pol sequences of various lengths and containing only two cleavage sites [33, 113, 195]. These systems have shown two independent sequential steps are required for the release of the mature PR. The first step is the intramolecular TF-PR cleavage that coincides with a large increase in mature-like enzyme activity [33, 113, 115] and the second is the intermolecular cleavage of PR-RT [195]. Accordingly, blocking cleavage at the N-terminus of PR also blocks the C-terminal cleavage of PR and results in a loss of infectivity [112, 114, 183, 204].

The order and timing of Gag precursor processing are well established with cleavage shown to occur in a sequential manner at primary, secondary and tertiary sites [142, 143] (Fig. 4). Recent analyses by Pettit *et al.* [141]

have now illuminated the order of Gag-Pol precursor cleavage. Using an *in vitro* system with full-length Gag-Pol the first cleavage of the Gag-Pol protein was shown to occur between p2 and NC to release MA-CA-P2 (42KD) and NC-TF-PR-RT-IN (120KD). Cleavage between P2 and NC is also the first processing event to occur in the Gag precursor protein [119, 142, 192]. The second cleavage site in Gag-Pol occurs within TF to release P6<sup>Pol</sup>-PR-RT-IN (113KD) and NC-TFP. Cleavage at this site has also been seen in infected cells [3, 26]. The Pettit *et al.* [141] study suggests that the release of PR is a relatively late event and that initial cleavages are carried out by PR intermediates (summarized in Fig. 4). PR processing of Gag and Gag-Pol is sequential [142, 173, 192] and it now appears that interactions at the dimeric interface of PR co-ordinate the order of precursor protein cleavage [141]. Work by Pettit *et al.* [141] work also supports previous investigations that suggest the initial cleavages are intramolecular as the order of Gag-Pol cleavage could be altered by an Ala to Pro change at codon position one of PR when cleavage was carried out by endogenous but not exogenous PR. Pettit *et al.* [140] have recently conducted an extensive analysis of the *in vitro* processing of full length Gag-Pol by it's embedded PR. Taking advantage of the fact that two active-site aspartic acids are required for an active PR, Pettit *et al.* [140] have shown via co-translation of various constructs containing PR-active site and/or processing-site mutations that cleavage requires an active PR to be present on the same Gag-Pol molecule as the cleavage site. This demonstration that the initial cleavages of the Gag-Pol precursor must be intramolecular was also supported by the finding that Gag-Pol associated PR was unable to cleave co-expressed Gag [140].

The position of PR between TF and RT appears critical for efficient processing and consequently viral budding and infectivity. When Gag-Pol constructs with an active PR replacing MA are co-transfected with Gag the released virus has defects in autoprocessing and in the *trans* processing of Gag [27, 187]. In addition, changes to the organisation of Pol proteins (PR-RT-IN) have been observed to prevent processing by PR [24]. While RT does not have a catalytic role in PR release [29, 195]; TF is clearly involved in PR regulation. However, the precise role of TF and the mechanism of regulation have not been determined. There is evidence to suggest that TF acts as a negative regulator of PR activity, as cleavage of TF from PR was found to be



**Fig. (4).** Schematic of the sequential processing in HIV-1 Gag and Gag-Pol. Triangles indicate cleavage sites. (A) Sequential processing of Gag adapted from [142]. (B) Sequential processing of Gag-Pol derived from [141]. Open triangles represent predicted intramolecular cleavages.

crucial for the stabilization of the PR dimeric structure [110]. In addition, TFP was shown to be a specific competitive inhibitor of the mature PR [138]. As a negative regulator of PR, TF may act to prevent premature processing, so that the Gag-Pol precursor remains intact until viral assembly is complete. Such a delay would have a number of advantages. For example, as the membrane targeting signal is in the N-terminus of Gag-Pol, delayed processing prevents precursor cleavage before Gag-Pol reaches the cell membrane for viral assembly [141]. Interestingly, TF may have an additional role that is not required for wild-type PR but comes into play in PR mutants with impaired PR dimerization, such as drug resistant PR mutants [37]. Dautin *et al.* [37] have shown that TF facilitated the folding and dimerization ability and enzyme activity of a PR variant with impaired dimer formation. Similarly, PR variants with dimer interface mutations can be compensated for when expressed as part of Gag-Pol [141].

### Viral Protein Processing and Particle Maturation

Sequential processing of the Gag protein appears critical for the maturation of HIV-1 from immature particles with a spherical capsid shell to mature particles with an electron dense cone shaped capsid core (for review see [120]). Investigation of the process of core maturation has linked each step with specific Gag cleavages [1, 64, 98, 192]. In the newly released immature particle MA-CA-p2-NC is attached to the viral membrane. The initial cleavage of Gag at P2/NC is essential for condensation of the electron dense core that contains the RNP, NC and the viral genomic RNA. MA-CA-p2 is then cleaved to release CA-p2 which leads to capsid condensation and the formation of a spherical sub-membrane shell. The mature conical core is finally generated following CA/p2 cleavage.

The maturation or “stabilization” of HIV-1’s RNA dimeric genome appears tightly coupled to proteolytic processing (for review see [132]). Dimeric RNA from PR negative “immature” particles displays an altered electrophoretic mobility and is less stable following heat treatment compared to wild-type particles [56]. Furthermore, maturation of the genomic RNA is dependent on the proteolytic release of the N-terminus of NC from the Gag precursor [164]. Using an *in vitro* transcription translation system Sheng *et al.* [166, 167] have demonstrated that cleavage of the Gag intermediate NCp15 requires the presence of RNA. In addition, HIV-1 mutants made defective in RNA dimerization by deletion of the DIS stem-loop show impaired protein processing [104]. Long-term culture of these mutants results in the appearance of compensatory mutations in *gag* that restore protein processing [105]. Deletion of the SL3 packaging signal that lies upstream of the Gag coding region generates defects in RNA packaging and dimerization [156] that can be rescued by compensatory mutations in *gag* (distinct from those described for the DIS-mutants) [153]. These mutations do not, however, restore RNA packaging and dimerization when introduced into a PR negative construct, suggesting the compensatory mutations act during the viral maturation process [153]. Overall, these studies highlight a complex and intricate relationship between genomic RNA dimer maturation and protein processing.

### THE MATURE VIRAL ENZYMES

The mature HIV-1 enzymes are multimeric proteins whose enzymatic activities are tightly linked to their tertiary structures. For detailed reviews of the structure and function of PR, RT, RNase H and IN see [36, 90, 93, 184]. The precise processes encompassing the expression of the Gag-Pol monomer, the release of the mature forms of the proteins and the conformational changes required for active multimeric enzymes have not been confirmed. This section will summarize our current understanding of these events.

### Maturation of the PR Homodimer

The mature HIV-1 PR protein is a homodimer whose dimerization is indispensable for catalytic activity as each subunit contributes a catalytic Asp to form the active site [109, 125]. The substrate binding cleft is positioned above the active site and the binding site entrance is covered by two flexible flaps (one from each monomer) that close down on the active site following substrate binding [128]. PR is stabilised at the dimer interface by a four stranded anti-parallel beta-sheet interaction between four interdigitating residues (residues 1-4 and 96-99) in the N- and C- termini of the two PR subunits [194]. Another structural feature important for the stability of the PR dimer is the complex scaffold of hydrogen bonds supporting the active site, termed the “fireman’s grip” [74, 176]. In HIV-1 the fireman’s grip is formed by the hydroxyl groups of two Thr residues in the active site Asp-Thr-Gly triplets. Other retroviral proteases, such as RSV and myeloblastosis-associated virus (MAV), have Ser instead of Thr in the active site triplet. Mutational analysis has shown that the use of Thr as opposed to Ser in this setting increases enzyme activity [96, 151, 154]. Recent analyses of the relative contributions of Thr or Ser to PR dimerization demonstrated that the use of Thr in the active site triplet made the PR dimer’s roughly 10 times more thermodynamically stable than their Ser-using counterparts [74]. Interestingly, Ingr *et al.* [74] also showed that the increased stability of the Thr-using PR’s was caused by a higher rate of dimer association than a slower rate of dimer dissociation. This suggests that the fireman’s grip plays an active role in PR dimerization, potentially orchestrating the initial interaction between the two PR monomers and aiding in the resolution of correct dimer conformation [74].

As PR is responsible for its own catalytic release from Gag-Pol and the first cleavages by PR appear to be intramolecular [33, 113, 115, 140, 141] there seems little doubt that the immediate precursor of the mature PR is the dimerized Gag-Pol precursor. The dimer interface accounts for >50% of the intermolecular contacts between the PR monomers [191, 194]. There are also multiple dimerization and oligomerization domains within Gag-Pol and assembly domains outside of PR contribute to PR dimer formation and catalytic activity [141]. In addition, there is no evidence for a naturally occurring PR monomer. The first description of a structure of the PR monomer utilised mutants that destabilise the PR dimer [76]. The tertiary fold of the PR monomer was similar to the individual subunits of the dimer, with exception of an exposed charged surface of the active site region and an open flap conformation [76]. It remains to be determined whether this folded structure is

reminiscent of the native PR domain within the Gag-Pol precursor as the structure of the Gag-Pol precursor in either monomeric or dimeric form has not been resolved. Identification of the structure of the Gag-Pol precursor will be invaluable in our understanding of the maturation of the viral enzymes as the first critical step in the maturation of PR, and consequently RT and IN, is the folding and dimerization of PR in the context of the Gag-Pol precursor protein.

### Maturation of the RT Heterodimer

The HIV-1 RT is an asymmetric heterodimer that consists of a large (560 amino acids, 66KDa: p66) and a small (440 amino acids, 51KDa: p51) subunit with common N termini. The C-terminal fragment that is removed from p66 to form p51 is the RNase H domain [42]. All enzyme activities reside in the p66 subunit of RT and p51 is catalytically inactive [100]. Accordingly, the role of p51 is primarily structural and the dimerization of p66 and p51 and the associated conformational changes are in fact required for enzyme function [44]. X-ray crystal structures of RT [71, 81, 95, 152] indicate a structure similar to a right hand with four subdomains, which are termed the finger, palm, thumb and connection domains. The p51 RT subunit contains the same polymerase subdomains as p66, yet their spatial arrangement is markedly different as p51 adopts a closed structure wherein the connection domain is rotated so that the active site residues are buried [95]. The RT heterodimer is the biologically relevant form of the enzyme. The monomeric subunits p66 and p51 display only low catalytic activity [150]. In studies using recombinant proteins the p66 homodimer, which is considered to be the immediate precursor to the RT heterodimer (see below), displays a level of RT catalytic activity similar to that of the heterodimer [150].

The accepted immediate precursor to the RT heterodimer is the p66 homodimer, although there is no published experimental data that directly proves this notion. This hypothesis is based on our knowledge of the structure of the HIV RT and gives a plausible explanation for the presence of a 1:1 ratio of p66 and p51 in the virion [188]. Since the p66 homodimer has two copies of RNase H, it is believed that due to steric constraints the RNase H domain in one of the p66 homodimer subunits must unravel to expose the protease cleavage site between the polymerase and RNase H domains [179]. The alternative to the sequential processing hypothesis is that the subunits are cleaved from various Gag-Pol molecules and subsequently interact to form the p66/p51 heterodimer and it is in fact possible to create a stable heterodimer by mixing p66 and p51 and purifying by chromatography [52]. To investigate the precursor to the RT heterodimer Sluis-Cremer *et al.* [170] have analyzed the processing kinetics of a 90Kd Pol fragment expressed in *E. Coli* and demonstrated that there was an accumulation of p66 prior to the appearance of p51. Two hours post-induction p66 and p51 were in equal amounts and it was possible to purify active RT from the lysate. In addition, the introduction of an L234 RT mutation that prevents RT dimer formation [61, 178] resulted in the appearance of only a small amount of p51 being produced that was then rapidly degraded, indicating that heterodimer formation was

dependent on RT's ability to dimerise [170]. Overall the results of this study support the premise that p66 is the immediate precursor to the RT heterodimer.

Tachedjian *et al.* (unpublished data) have also investigated whether p66 is the immediate RT heterodimer precursor, exploiting mutations near the RT dimer interface that have been shown to inhibit RT heterodimerization [177] and p66 homodimerization (Tachedjian *et al.* unpublished data) in yeast two-hybrid and *in vitro* binding assays. These mutations led to the production of virus particles with no defects in Gag processing, Gag-Pol stability or Gag-Pol incorporation in the virion. However, these viral particles were noninfectious and devoid of virion associated RT activity as a result of the specific instability of the RT. Analysis of cell lysates transfected with mutant virus demonstrated decreased levels of p66 and undetectable levels of p51 compared to wild-type. It appears that the mutations are inhibiting the homodimerization of p66 and that this form of the RT is unstable and highly susceptible to proteolysis by the HIV protease. These data support the hypothesis that the p66 homodimer is the immediate RT heterodimer precursor.

### Maturation of the IN Tetramer

While the basic chemical process of integration is well understood the structure of the mature active IN and the complex it forms with the substrate remain elusive. IN's 228 residues make up three independent domains; an N-terminal domain that is characterised by a HH-CC zinc binding motif [201], a catalytic core domain that contains the essential residues D64, D116 and E152 and a C-terminal domain that facilitates DNA binding. Notably, the catalytic core domain is reminiscent of the RNase H structure of HIV-1 RT and the two have catalytic acidic residues in similar positions in the enzyme active sites [47]. Although the full length IN has not been crystallized, the structures of the three individual domains as well as paired domains have been solved [15, 25, 47, 48, 108, 189]. The structural studies indicate that IN forms a dimer. However, the mature active form of IN is thought to be at least a tetramer [49, 83, 201]. The catalytic core domain contains a region which forms a strong dimeric interface and in the solution of the paired N-terminal and catalytic core domains there is evidence for dimeric interactions in the N-terminal domain [84]. However, in the dimer the spacing between the active sites (30°A) is not compatible with the spacing required to insert two strands of DNA (15°A) [15, 184]. It is unclear whether an IN dimer is in fact active or if, as suggested by modelling the independently solved structures, the catalytic site is formed when two active sites come together from interacting dimers to galvanize the functional tetrameric enzyme [15, 145, 184]

## THE VIRAL ENZYMES AS ANTI-HIV DRUG TARGETS

It is now two decades since AIDS was first recognized and HIV-1 identified as the causal agent [127]. The recent licensing of "Fuzeon" (enfuvirtide), a fusion inhibitor that blocks HIV entry into CD4+ T-cells marked the arrival of the first anti-HIV drug not targeted at RT or PR. When used in combination in highly active antiretroviral therapy

(HAART) the nucleoside and nonnucleoside RT inhibitors and PR inhibitors have been successful in delaying the onset of AIDS. However, drug toxicity which inevitably leads to lipodystrophy and metabolic disorders and the unavoidable development of resistance mutations has made understanding the action of these inhibitors and the identification of new targets essential.

Drugs targeting RT belong to two main classes, the nucleoside reverse transcriptase inhibitors (NRTIs) and the non-nucleoside reverse transcriptase inhibitors (NNRTIs). The NRTIs are analogs of the natural substrates of the RT and become incorporated into the nascent DNA strand during reverse transcription preventing chain elongation. NNRTIs inhibit RT by binding to an allosteric site on the polymerase domain of the p66 subunit. Our understanding of how these inhibitors work is continuing to grow. In particular there is new evidence revealing the mechanism of NNRTIs ([179] and Tachedjian *et al.* unpublished). The recognized mechanism of action of NNRTIs is to inhibit the mature RT heterodimer by binding to a hydrophobic pocket in the p66 RT subunit, which is proximal to the polymerase active site. Targeting the RT in this way leads to inhibition of reverse transcription, which is an early step in the virus life cycle. The recent demonstration of enhancement of RT heterodimerization [180] and in particular p66 homodimerization (Tachedjian *et al.* unpublished) by NNRTIs raises the possibility that these drugs might act at the late stage of the virus life cycle, namely at activation of PR and Gag-Pol processing. In support of this notion, efavirenz treatment of cells transfected with an infectious HIV molecular clone altered intracellular processing of Gag-Pol, suggesting more efficient cleavage of the mature proteins. Furthermore, a significant reduction in viral particle release in efavirenz treated cells without a concomitant decrease in the expression of intracellular HIV-1 CA was observed. Importantly, the concentrations of drug used were at levels normally seen in patients treated with efavirenz and were not toxic. The decrease in viral particle release conferred by efavirenz was PR dependent since no decrease was observed in the presence of drug when cells were transfected with a PR negative HIV-1 clone. NNRTIs that are weak or do not enhance RT heterodimerization failed to inhibit viral particle release indicating that the effect is related to enhancement of RT dimerization. Overall it appears that efavirenz enhances the interaction of Gag-Pol in the cell through p66, which leads to premature activation of the HIV-1 PR (Tachedjian *et al.* unpublished).

The "other" viral enzymes, RNase H and IN, with their critical role in viral replication are excellent anti-HIV-1 targets. Many inhibitors of IN have been described (for review see [146]), and with the successful screening of natural inhibitors of IN there has been a recent flurry of papers describing a range of structurally diverse fungal metabolites that are potent inhibitors of IN activity [82, 168]. Perhaps the most promising of the IN inhibitors to date are the 1, 3 diketo acids (DKA) which specifically inhibit the strand transfer step of integration and have demonstrated anti-viral activity in cell-culture [67]. In fact the diketo analogue S-1360 has progressed to the point of clinical trials in HIV-1 seropositive patients [8]. Not surprisingly resistance develops to DKAs in cell culture after approximately 30 weeks [51]. However, as the resistant

mutants remain sensitive to RT and PR inhibitors as well as the 5H-pyrano[2, 3-d:-6, 5-d']dipyrimidine molecule V-165, a novel IN inhibitor [133], the DKAs will be a welcome addition to the combination therapies currently used against HIV-1 [51]. In contrast there have been very few descriptions of viable inhibitors of RNase H (for review see [93]), although large scale screening is ongoing [137]. Recently, a novel DKA has been shown to selectively bind and inhibit RNase H [161]. As this DKA is metal dependent it has been suggested to interact with the metals in the RNase H active site. This is analogous to the mechanism of action proposed for HIV-1 IN inhibitors of the same structural class [67], although the IN inhibitors do not inhibit RNase H.

Structural studies have played an important role in the development of inhibitors for PR and RT and continue to facilitate the identification of new classes of anti-HIV drugs. For example, structure based design is being used to develop new inhibitors of RT that function by a different mechanism than the current inhibitors and can therefore form part of a multi-faceted approach [169]. Moreover, as the function of the viral enzymes is tied to their multimeric conformations, interference with the protein-protein interactions of PR, RT and IN makes an attractive target for blocking HIV-1 replication, and many small molecule inhibitors have been identified to inhibit these interactions (for a review of this approach see [171]). Notably, as RNase H and IN are structurally similar, small molecule antagonists of IN may also target RNase H and vice versa. Accordingly, oligodeoxynucleotide aptamers that inhibit RNase H also hinder IN activity [38]. Current inhibitors of PR directly target the PR active site. Alternative methods of inhibition, such as preventing or interfering with PR dimer formation are being pursued. Dimerization inhibitors have been previously described [19, 160] and this approach will be aided by the recent advances in defining the structure of the PR monomer [75, 76, 111]. Structural analysis directed towards determining the conformation of the full length Gag-Pol polyprotein may also prove particularly useful, enabling us to target the viral enzymes in their immature forms.

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## REFERENCES

- [1] Accola MA, Hoglund S, Gottlinger HG. (1998). *Journal of Virology*. 72: 2072-2078.
- [2] Alam SL, Wills NM, Ingram JA, Atkins JF, Gesteland RF. (1999). *Journal of Molecular Biology*. 288: 837-852.
- [3] Almog N, Roller R, Arad G, Passi-Even L, Wainberg MA, Kotler M. (1996). *Journal of Virology*. 70: 7228-7232.
- [4] Ansari-Lari M, Gibbs R. (1996). *Journal of Virology*. 70: 3870-3875.
- [5] Arrigo SJ, Huffman K. (1995). *Journal of Virology*. 69: 5988-5994.
- [6] Baril M, Dulude D, Gendron K, Lemay G, Brakier-Gingras L. (2003). *RNA*. 9: 1246-1253.

- [7] Bidou L, Stahl G, Grima B, Liu H, Cassan M, Rousset JP. (1997). RNA. 3: 1153-1158.
- [8] Billich A. (2003). Current Opinion in Investigational Drugs. 4: 206-209.
- [9] Biswas P, Jiang X, Pacchia AL, Dougherty JP, Peltz SW. (2004). Journal of Virology. 78: 2082-2087.
- [10] Buchschacher GL, Jr., Yu L, Murai F, Friedmann T, Miyanoahara A. (1999). Journal of Virology. 73: 9632-9637.
- [11] Bukrinsky MI, Haggerty S, Dempsey MP, Sharova N, Adzhubel A, Spitz L, Lewis P, Goldfarb D, Emerman M, Stevenson M. (1993). Nature. 365: 666-669.
- [12] Bukrinsky MI, Sharova N, Dempsey MP, Stanwick TL, Bukrinskaya AG, Haggerty S, Stevenson M. (1992). Proceedings of the National Academy of Sciences USA. 89: 6580-6584.
- [13] Bukrinsky MI, Sharova N, McDonald TL, Pushkarskaya T, Tarpley WG, Stevenson M. (1993). Proceedings of the National Academy of Sciences USA. 90: 6125-6129.
- [14] Burniston MT, Cimarelli A, Colgan J, Curtis SP, Luban J. (1999). Journal of Virology. 73: 8527-8540.
- [15] Cai M, Zheng R, Caffrey M, Craigie R, Clore GM, Gronenborn AM. (1997). Nature Structural Biology. 4: 567-577.
- [16] Campbell S, Rein A. (1999). Journal of Virology. 73: 2270-2279.
- [17] Campbell S, Vogt VM. (1995). Journal of Virology. 69: 6487-6497.
- [18] Campbell S, Vogt VM. (1997). Journal of Virology. 71: 4425-4435.
- [19] Cartas M, Singh SP, Serio D, Rizvi TA, Kalyanaraman VS, Goldsmith CS, Zaki SR, Weber IT, Srinivasan A. (2001). DNA and Cell Biology. 20: 797-805.
- [20] Cassan M, Berteaux V, Angrand PO, Rousset JP. (1990). Research in Virology. 141: 597-610.
- [21] Cassan M, Delaunay N, Vaquero C, Rousset JP. (1994). Journal of Virology. 68: 1501-1508.
- [22] Cen S, Niu M, Saadatmand J, Guo F, Huang Y, Nabel GJ, Kleiman L. (2004). Journal of Virology. 78: 1042-1049.
- [23] Chang SY, Sutthent R, Auewarakul P, Apichartpiyakul C, Essex M, Lee TH. (1999). AIDS Research and Human Retroviruses. 15: 1591-1596.
- [24] Chang YY, Yu SL, Syu WJ. (1999). Journal of Biomedical Science. 6: 333-341.
- [25] Chen JC, Krucinski J, Miercke LJ, Finer-Moore JS, Tang AH, Leavitt AD, Stroud RM. (2000). Proceedings of the National Academy of Sciences USA. 97: 8233-8238.
- [26] Chen N, Morag A, Almog N, Blumenzweig I, Dreazin O, Kotler M. (2001). Journal of General Virology. 82: 581-590.
- [27] Chen S-W, Chiu H-C, Liao W-H, Wang F-D, Chen SS-L, Wang C-T. (2004). Virology. 318: 534-541.
- [28] Chen YL, Ts'ai PW, Yang CC, Wang CT. (1997). Journal of General Virology. 78 (Pt 10): 2497-2501.
- [29] Cherry E, Liang C, Rong L, Quan Y, Inouye P, Li X, Morin N, Kotler M, Wainberg MA. (1998). Journal of Molecular Biology. 284: 43-56.
- [30] Chiu H-C, Chen S-W, Liao W-H, Wang C-T. (2004). Journal of Biomedical Sciences. 11: 398-407.
- [31] Chiu H-C, Yao S-Y, Wang C-T. (2002). Journal of Virology. 76: 3221-3231.
- [32] Cimarelli A, Sandin S, Hoglund S, Luban J. (2000). Journal of Virology. 74: 3046-3057.
- [33] Co E, Koelsch G, Lin Y, Ido E, Hartsuck JA, Tang J. (1994). Biochemistry. 33: 1248-1254.
- [34] Cosson P. (1996). EMBO Journal. 15: 5783-5788.
- [35] Costa LJ, Zheng Y-H, Sabotic J, Mak J, Fackler OT, Peterlin BM. (2004). Journal of Virology. 78: 5311-5323.
- [36] Craigie R. (2001). Journal of Biological Chemistry. 276: 23213-23216.
- [37] Dautin N, Karimova G, Ladant D. (2003). Journal of Virology. 77: 8216-8226.
- [38] de Soultrait VR, Lozach PY, Altmeyer R, Tarrago-Litvak L, Litvak S, Andreola ML. (2002). Journal of Molecular Biology. 324: 195-203.
- [39] Depienne C, Roques P, Creminon C, Fritsch L, Casseron R, Dormont D, Dargemont C, Benichou S. (2000). Experimental Cell Research. 260: 387-395.
- [40] Derdowski A, Ding L, Spearman P. (2004). Journal of Virology. 78: 1230-1242.
- [41] Dettenhofer M, Yu XF. (1999). Journal of Virology. 73: 4696-4704.
- [42] di Marzo Veronese F, Copeland TD, DeVico AL, Rahman R, Oroszlan S, Gallo RC, Sarngadharan MG. (1986). Science. 231: 1289-1291.
- [43] Dinman J, Richter S, Plant E, Taylor R, Hammell A, Rana T. (2002). Proceedings of the National Academy of Sciences USA. 99: 5331-5336.
- [44] Divita G, Rittinger K, Geourjon C, Deleage G, Goody RS. (1995). Journal of Molecular Biology. 245: 508-521.
- [45] Dulude D, Baril M, Brakier-Gingras L. (2002). Nucleic Acids Research. 30: 5094-5102.
- [46] Dupont S, Sharova N, DeHoratius C, Virbasius CM, Zhu X, Bukrinskaya AG, Stevenson M, Green MR. (1999). Nature. 402: 681-685.
- [47] Dyda F, Hickman AB, Jenkins TM, Engelman A, Craigie R, Davies DR. (1994). Science. 266: 1981-1986.
- [48] Eijkelenboom AP, Lutzke RA, Boelens R, Plasterk RH, Kaptein R, Hard K. (1995). Nature Structural Biology. 2: 807-810.
- [49] Esposito D, Craigie R. (1999). Advances in Virus Research. 52: 319-333.
- [50] Fackler OT, d'Aloja P, Baur AS, Federico M, Peterlin BM. (2001). Journal of Virology. 75: 6601-6608.
- [51] Fikkert V, Van Maele B, Vercammen J, Hantson A, Van Remoortel B, Michiels M, Gurnari C, Pannecouque C, De Maeyer M, Engelborghs Y, De Clercq E, Debyser Z, Witvrouw M. (2003). Journal of Virology. 77: 11459-11470.
- [52] Fletcher RS, Holleschak G, Nagy E, Arion D, Borkow G, Gu Z, Wainberg MA, Parniak MA. (1996). Protein Expression and Purification. 7: 27-32.
- [53] Fouchier RA, Meyer BE, Simon JH, Fischer U, Albright AV, Gonzalez-Scarano F, Malim MH. (1998). Journal of Virology. 72: 6004-6013.
- [54] Freed EO. (2003). Trends in Microbiology. 11: 56-59.
- [55] Freed EO, Martin MA. (1995). Journal of Virology. 69: 1984-1989.
- [56] Fu W, Gorelick RJ, Rein A. (1994). Journal of Virology. 68: 5013-5018.
- [57] Gallay P, Hope T, Chin D, Trono D. (1997). Proceedings of the National Academy of Sciences USA. 94: 9825-9830.
- [58] Gao K, Gorelick RJ, Johnson DG, Bushman F. (2003). Journal of Virology. 77: 1598-1603.
- [59] Garrus JE, von Schwedler UK, Pornillos OW, Morham SG, Zavitz KH, Wang HE, Wettstein DA, Stray KM, Cote M, Rich RL, Myszka DG, Sundquist WI. (2001). Cell. 107: 55-65.
- [60] Gheysen D, Jacobs E, de Foresta F, Thiriart C, Francotte M, Thines D, De Wilde M. (1989). Cell. 59: 103-112.
- [61] Ghosh R, Jacques PS, Rodgers DW, Ottman M, Darlix JL, Le Grice SF. (1996). Biochemistry. 35: 8553-8562.
- [62] Goff SP. (2004). Cell Cycle. 3: 123-125.
- [63] Gottlinger HG, Dorfman T, Sodroski JG, Haseltine WA. (1991). Proceedings of the National Academy of Sciences USA. 88: 3195-3199.
- [64] Gross I, Hohenberg H, Wilk T, Wieggers K, Grattinger M, Muller B, Fuller S, Krausslich HG. (2000). EMBO Journal. 19: 103-113.
- [65] Halwani R, Khorchid A, Cen S, Kleiman L. (2003). Journal of Virology. 77: 3973-3984.
- [66] Hatfield DL, Levin JG, Rein A, Oroszlan S. (1992). Advances in Virus Research. 41: 193-239.
- [67] Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, Espeseth A, Gabryelski L, Schleif W, Blau C, Miller MD. (2000). Science. 287: 646-50.
- [68] Heinzinger NK, Bukinsky MI, Haggerty SA, Ragland AM, Kewalramani V, Lee MA, Gendelman HE, Ratner L, Stevenson M, Emerman M. (1994). Proceedings of the National Academy of Sciences USA. 91: 7311-7315.
- [69] Hill MK, Hooker CW, Harrich D, Crowe SM, Mak J. (2001). Journal of Virology. 75: 6835-6840.
- [70] Hill MK, Shehu-Xhilaga M, Crowe SM, Mak J. (2002). Journal of Virology. 76: 11245-11253.
- [71] Huang H, Chopra R, Verdine GL, Harrison SC. (1998). Science. 282: 1669-1675.
- [72] Huang M, Martin MA. (1997). Journal of Virology. 71: 4472-4478.
- [73] Huang M, Orenstein JM, Martin MA, Freed EO. (1995). Journal of Virology. 69: 6810-6818.
- [74] Ingr M, Uhlíkova T, Strisovsky K, Majerova E, Konvalinka J. (2003). Protein Science. 12: 2173-2182.

- [75] Ishima R, Ghirlando R, Tozser J, Gronenborn AM, Torchia DA, Louis JM. (2001). *Journal of Biological Chemistry*. 276: 49110-49116.
- [76] Ishima R, Torchia DA, Lynch SM, Gronenborn AM, Louis JM. (2003). *Journal of Biological Chemistry*. 278: 43311-43319.
- [77] Jacks T. (1990). *Current Topics in Microbiology and Immunology*. 157: 93-124.
- [78] Jacks T, Madhani HD, Masiarz FR, Varmus HE. (1988). *Cell*. 55: 447-458.
- [79] Jacks T, Power MD, Masiarz FR, Luciw PA, Barr PJ, Varmus HE. (1988). *Nature*. 331: 280-283.
- [80] Jacks T, Varmus HE. (1985). *Science*. 230: 1237-1242.
- [81] Jacobo-Molina A, Ding J, Nanni RG, Clark AD, Jr., Lu X, Tantillo C, Williams RL, Kamer G, Ferris AL, Clark P, Hizi A, Hughes SH, Arnold E. (1993). *Proceedings of the National Academy of Sciences USA*. 90: 6320-6324.
- [82] Jayasuriya H, Guan Z, Polishook JD, Dombrowski AW, Felock PJ, Hazuda DJ, Singh SB. (2003). *Journal of Natural Products*. 66: 551-553.
- [83] Jenkins TM, Engelman A, Ghirlando R, Craigie R. (1996). *Journal of Biological Chemistry*. 271: 7712-7718.
- [84] Jenkins TM, Hickman AB, Dyda F, Ghirlando R, Davies DR, Craigie R. (1995). *Proceedings of the National Academy of Sciences USA*. 92: 6057-6061.
- [85] Kang H. (1998). *Biochimica Et Biophysica Acta*. 1397: 73-78.
- [86] Kaplan AH, Manchester M, Swanstrom R. (1994). *Journal of Virology*. 68: 6782-6786.
- [87] Kaplan AH, Zack JA, Knigge M, Paul DA, Kempf DJ, Norbeck DW, Swanstrom R. (1993). *Journal of Virology*. 67: 4050-4055.
- [88] Karacostas V, Wolfje EJ, Nagashima K, Gonda MA, Moss B. (1993). *Virology*. 193: 661-671.
- [89] Katoh I, Yoshinaka Y, Rein A, Shibuya M, Odaka T, Oroszlan S. (1985). *Virology*. 145: 280-292.
- [90] Katz RA, Skalka AM. (1994). *Annual Review of Biochemistry*. 63: 133-173.
- [91] Khorchid A, Halwani R, Wainberg MA, Kleiman L. (2002). *Journal of Virology*. 76: 4231-4137.
- [92] Khorchid A, Javanbakht H, Wise S, Halwani R, Parniak MA, Wainberg MA, Kleiman L. (2000). *Journal of Molecular Biology*. 299: 17-26.
- [93] Klarmann GJ, Hawkins ME, Le Grice SF. (2002). *AIDS Reviews*. 4: 183-194.
- [94] Kohl NE, Emini EA, Schleif WA, Davis LJ, Heimbach JC, Dixon RA, Scolnick EM, Sigal IS. (1988). *Proceedings of the National Academy of Sciences USA*. 85: 4686-4690.
- [95] Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA. (1992). *Science*. 256: 1783-1790.
- [96] Konvalinka J, Litterst MA, Welker R, Kottler H, Rippmann F, Heuser AM, Krausslich HG. (1995). *Journal of Virology*. 69: 7180-7186.
- [97] Krausslich HG. (1991). *Proceedings of the National Academy of Sciences USA*. 88: 3213-3217.
- [98] Krausslich HG, Facke M, Heuser AM, Konvalinka J, Zentgraf H. (1995). *Journal of Virology*. 69: 3407-3419.
- [99] Larson DR, Ma YM, Vogt VM, Webb WW. (2003). *Journal of Cell Biology*. 162: 1233-1244.
- [100] Le Grice SF, Naas T, Wohlgensinger B, Schatz O. (1991). *EMBO Journal*. 10: 3905-3911.
- [101] Le SY, Chen JH, Maizel JV. (1989). *Nucleic Acids Research*. 17: 6143-6152.
- [102] Lee YM, Yu XF. (1998). *Virology*. 243: 78-93.
- [103] Levin JG, Hattfield DL, Oroszian S, Rein A. (1993). Mechanisms of translational suppression used in the biosynthesis of reverse transcriptase. In: Skalka AM, Goff SP Eds, *Reverse Transcriptase*. Cold Spring Harbor, Cold Spring Harbor Laboratory Press. pp. 5-31.
- [104] Liang C, Rong L, Cherry E, Kleiman L, Laughrea M, Wainberg MA. (1999). *Journal of Virology*. 73: 6147-6151.
- [105] Liang C, Rong L, Laughrea M, Kleiman L, Wainberg MA. (1998). *Journal of Virology*. 72: 6629-6636.
- [106] Lingappa JR, Hill RL, Wong ML, Hegde RS. (1997). *Journal of Cell Biology*. 136: 567-581.
- [107] Liu H, Wu X, Xiao H, Conway J, Kappes J. (1997). *Journal of Virology*. 71: 7704-7710.
- [108] Lodi PJ, Ernst JA, Kuszewski J, Hickman AB, Engelman A, Craigie R, Clore GM, Gronenborn AM. (1995). *Biochemistry*. 34: 9826-9833.
- [109] Loeb DD, Hutchison CA, 3rd, Edgell MH, Farmerie WG, Swanstrom R. (1989). *Journal of Virology*. 63: 111-121.
- [110] Louis JM, Clore GM, Gronenborn AM. (1999). *Nature Structural Biology*. 6: 868-875.
- [111] Louis JM, Ishima R, Nesheiwat I, Pannell LK, Lynch SM, Torchia DA, Gronenborn AM. (2003). *Journal of Biological Chemistry*. 278: 6085-6092.
- [112] Louis JM, McDonald RA, Nashed NT, Wondrak EM, Jerina DM, Oroszlan S, Mora PT. (1991). *European Journal of Biochemistry*. 199: 361-369.
- [113] Louis JM, Nashed NT, Parris KD, Kimmel AR, Jerina DM. (1994). *Proceedings of the National Academy of Sciences USA*. 91: 7970-7974.
- [114] Louis JM, Oroszlan S, Mora PT. (1991). *Advances in Experimental Medicine and Biology*. 306: 499-502.
- [115] Louis JM, Wondrak EM, Kimmel AR, Wingfield PT, Nashed NT. (1999). *Journal of Biological Chemistry*. 274: 23437-23442.
- [116] Mammano F, Kondo E, Sodroski J, Bukovsky A, Gottlinger HG. (1995). *Journal of Virology*. 69: 3824-3830.
- [117] Martin-Serrano J, Zang T, Bieniasz PD. (2001). *Nature Medicine*. 7: 1313-1319.
- [118] Mergener K, Facke M, Welker R, Brinkmann V, Gelderblom HR, Krausslich HG. (1992). *Virology*. 186: 25-39.
- [119] Mervis RJ, Ahmad N, Lillehoj EP, Raum MG, Salazar FH, Chan HW, Venkatesan S. (1988). *Journal of Virology*. 62: 3993-4002.
- [120] Morikawa Y. (2003). *Current HIV Research*. 1: 1-14.
- [121] Morikawa Y, Goto T, Sano K. (1999). *Journal of Biological Chemistry*. 274: 27997-8002.
- [122] Murakami T, Ablan S, Freed EO, Tanaka Y. (2004). *Journal of Virology*. 78: 1026-1031.
- [123] Murakami T, Freed EO. (2000). *Journal of Virology*. 74: 3548-3554.
- [124] Muriaux D, Mirro J, Harvin D, Rein A. (2001). *Proceedings of the National Academy of Sciences USA*. 98: 5246-5251.
- [125] Navia MA, Fitzgerald PM, McKeever BM, Leu CT, Heimbach JC, Herber WK, Sigal IS, Darke PL, Springer JP. (1989). *Nature*. 337: 615-620.
- [126] Nermut MV, Zhang W-H, Francis G, Ciampor F, Morikawa Y, Jones IM. (2003). *Virology*. 305: 219-227.
- [127] Nicholls EH. (2004). *Endeavour*. 28: 1.
- [128] Nicholson LK, Yamazaki T, Torchia DA, Grzesiek S, Bax A, Stahl SJ, Kaufman JD, Wingfield PT, Lam PY, Jadhav PK, Hodge N, Domaille PJ, Chang C-H (1995). *Nature Structural Biology*. 2: 274-280.
- [129] Olivetta E, Pugliese K, Bona R, D'Aloja P, Ferrantelli F, Santarcangelo AC, Mattia G, Verani P, Federico M. (2000). *Journal of Virology*. 74: 483-492.
- [130] Orlova M, Yueh A, Leung J, Goff SP. (2003). *Cell*. 115: 319-331.
- [131] Ott DE, Coren LV, Chertova EN, Gagliardi TD, Nagashima K, Sowder II RC, Poon DTK, Gorelick RJ. (2003). *Journal of Virology*. 77: 5547-5556.
- [132] Paillart JC, Shehu-Xhilaga M, Marquet R, Mak J. (2004). *Nature Reviews Microbiology*. 2: 461-472.
- [133] Pannecouque C, Pluyers W, Van Maele B, Tetz V, Cherepanov P, De Clercq E, Witvrouw M, Debyser Z. (2002). *Current Biology*. 12: 1169-1177.
- [134] Park J, Morrow CD. (1991). *Journal of Virology*. 65: 5111-5117.
- [135] Park J, Morrow CD. (1992). *Journal of Virology*. 66: 6304-6313.
- [136] Parkin NT, Chamorro M, Varmus HE. (1992). *Journal of Virology*. 66: 5147-5151.
- [137] Parniak MA, Min KL, Budihas SR, Le Grice SF, Beutler JA. (2003). *Analytical Biochemistry*. 322: 33-39.
- [138] Paulus C, Hellebrand S, Tessmer U, Wolf H, Krausslich HG, Wagner R. (1999). *Journal of Biological Chemistry*. 274: 21539-21543.
- [139] Paxton W, Connor R, Landau N. (1993). *Journal of Virology*. 67: 7229-7237.
- [140] Pettit SC, Everitt LE, Choudhury S, Dunn BM, Kaplan AH. (2004). *Journal of Virology*. 78: 8477-8485.
- [141] Pettit SC, Gulnik S, Everitt L, Kaplan AH. (2003). *Journal of Virology*. 77: 366-374.
- [142] Pettit SC, Moody MD, Wehbie RS, Kaplan AH, Nantermet PV, Klein CA, Swanstrom R. (1994). *Journal of Virology*. 68: 8017-8027.
- [143] Pettit SC, Sheng N, Tritch R, Erickson-Viitanen S, Swanstrom R. (1998). *Advances in Experimental Medicine and Biology*. 436: 15-25.

- [144] Pluymer W, Cherepanov P, Schols D, De Clercq E, Debysers Z. (1999). *Virology*. 258: 327-332.
- [145] Podtelezhnikov AA, Gao K, Bushman FD, McCammon JA. (2003). *Biopolymers*. 68: 110-120.
- [146] Pommier Y, Marchand C, Neamati N. (2000). *Antiviral Research*. 47: 139-148.
- [147] Ratner L, Haseltine W, Patarca R, Livak KJ, Starcich B, Josephs SF, Doran ER, Rafalski JA, Whitehorn EA, Baumeister K, Ivanoff L, Peteway Jr, SR, Pearson ML, Lautenberger JA, Papas TS, Ghrayeb J, Chang NT, Gallo RC, Wong-Staal F. (1985). *Nature*. 313: 277-284.
- [148] Reil H, Bukovsky AA, Gelderblom HR, Gottlinger HG. (1998). *EMBO Journal*. 17: 2699-2708.
- [149] Reil H, Kollmus H, Weidle UH, Hauser H. (1993). *Journal of Virology*. 67: 5579-5584.
- [150] Restle T, Muller B, Goody R. (1990). *Journal of Biological Chemistry*. 265: 8986-8988.
- [151] Ridky TW, Bizub-Bender D, Cameron CE, Weber IT, Wlodawer A, Copeland T, Skalka AM, Leis J. (1996). *Journal of Biological Chemistry*. 271: 10538-10544.
- [152] Rodgers DW, Gamblin SJ, Harris BA, Ray S, Culp JS, Hellmig B, Woolf DJ, Debouck C, Harrison SC. (1995). *Proceedings of the National Academy of Sciences USA*. 92: 1222-1226.
- [153] Rong L, Russell RS, Hu J, Laughrea M, Wainberg MA, Liang C. (2003). *Virology*. 314: 221-228.
- [154] Rose JR, Babe LM, Craik CS. (1995). *Journal of Virology*. 69: 2751-8.
- [155] Ross EK, Fuerst TR, Orenstein JM, O'Neill T, Martin MA, Venkatesan S. (1991). *AIDS Research and Human Retroviruses*. 7: 475-483.
- [156] Russell RS, Hu J, Beriault V, Moulard AJ, Kleiman L, Wainberg MA, Liang C. (2003). *Journal of Virology*. 77: 84-96.
- [157] Sandefur S, Smith RM, Varthakavi V, Spearman P. (2000). *Journal of Virology*. 74: 7238-7249.
- [158] Schatzl H, Gelderblom HR, Nitschko H, von der Helm K. (1991). *Archives of Virology*. 120: 71-81.
- [159] Scheifele LZ, Garbitt RA, Rhoads JD, Parent LJ. (2002). *Proceedings of the National Academy of Sciences USA*. 99: 3944-3949.
- [160] Schramm HJ, Billich A, Jaeger E, Rucknagel KP, Arnold G, Schramm W. (1993). *Biochemical And Biophysical Research Communications*. 194: 595-600.
- [161] Shaw-Reid CA, Munshi V, Graham P, Wolfe A, Witmer M, Danzeisen R, Olsen DB, Carroll SS, Embrey M, Wai JS, Miller MD, Cole JL, Hazuda DJ. (2003). *Journal of Biological Chemistry*. 278: 2777-2780.
- [162] Shehu-Xhilaga M, Crowe SM, Mak J. (2001). *Journal of Virology*. 75: 1834-1841.
- [163] Shehu-Xhilaga M, Hill M, Marshall J, Kappes J, Crowe SM, Mak J. (2002). *Journal of Virology*. 76: 4331-4340.
- [164] Shehu-Xhilaga M, Krausslich HG, Pettit S, Swanstrom R, Lee JY, Marshall JA, Crowe SM, Mak J. (2001). *Journal of Virology*. 75: 9156-9164.
- [165] Shen LX, Tinoco I, Jr. (1995). *Journal of Molecular Biology*. 247: 963-978.
- [166] Sheng N, Erickson-Viitanen S. (1994). *Journal of Virology*. 68: 6207-6214.
- [167] Sheng N, Pettit SC, Tritch RJ, Ozturk DH, Rayner MM, Swanstrom R, Erickson-Viitanen S. (1997). *Journal of Virology*. 71: 5723-5732.
- [168] Singh SB, Jayasuriya H, Dewey R, Polishook JD, Dombrowski AW, Zink DL, Guan Z, Collado J, Platas G, Pelaez F, Felock PJ, Hazuda DJ. (2003). *Journal of Industrial Microbiology and Biotechnology*. 30: 721-731.
- [169] Skillman AG, Maurer KW, Roe DC, Stauber MJ, Eargle D, Ewing TJ, Muscate A, Davioud-Charvet E, Medaglia MV, Fisher RJ, Arnold E, Gao HQ, Buckheit R, Boyer PL, Hughes SH, Kuntz ID, Kenyon GL. (2002). *Bioorganic Chemistry*. 30: 443-458.
- [170] Sluis-Cremer N, Arion D, Abram ME, Parniak MA. (2004). *International Journal Of Biochemistry and Cell Biology*. 36: 1836-1847.
- [171] Sluis-Cremer N, Tachedjian G. (2002). *European Journal of Biochemistry*. 269: 5103-5111.
- [172] Smith AJ, Srinivasakumar N, Hammarskjold ML, Rekosh D. (1993). *Journal of Virology*. 67: 2266-2275.
- [173] Speck RR, Flexner C, Tian C-J, Yu X-F. (2000). *Antimicrobial Agents and Chemotherapy*. 44: 1397-1403.
- [174] Srinivasakumar N, Hammarskjold ML, Rekosh D. (1995). *Journal of Virology*. 69: 6106-6114.
- [175] Staple DW, Butcher SE. (2003). *Nucleic Acids Research*. 31: 4326-4331.
- [176] Strisovsky K, Tessmer U, Langner J, Konvalinka J, Krausslich HG. (2000). *Protein Science*. 9: 1631-1641.
- [177] Tachedjian G, Aronson HE, de los Santos M, Seehra J, McCoy JM, Goff SP. (2003). *Journal of Molecular Biology*. 326: 381-396.
- [178] Tachedjian G, Aronson HE, Goff SP. (2000). *Proceedings of the National Academy of Sciences USA*. 97: 6334-6339.
- [179] Tachedjian G, Goff SP. (2003). *Current Opinion in Investigational Drugs*. 4: 966-973.
- [180] Tachedjian G, Orlova M, Sarafianos SG, Arnold E, Goff SP. (2001). *Proceedings of the National Academy of Sciences USA*. 98: 7188-7193.
- [181] Tang C, Loeliger E, Luncsford P, Kinde I, Beckett D, Summers MF. (2004). *Proceedings of the National Academy of Sciences USA*. 101: 517-522.
- [182] Telenti A, Martinez R, Munoz M, Bleiber G, Greub G, Sanglard D, Peters S. (2002). *Journal of Virology*. 76: 7868-7873.
- [183] Tessmer U, Krausslich HG. (1998). *Journal of Virology*. 72: 3459-3463.
- [184] Turner BG, Summers MF. (1999). *Journal of Molecular Biology*. 285: 1-32.
- [185] VerPlank L, Bouamr F, LaGrassa TJ, Agresta B, Kikonyogo A, Leis J, Carter CA. (2001). *Proceedings of the National Academy of Sciences USA*. 98: 7724-7729.
- [186] Wain-Hobson S, Sonigo P, Danos O, Cole S, Alizon M. (1985). *Cell*. 40: 9-17.
- [187] Wang CT, Chou YC, Chiang CC. (2000). *Journal of Virology*. 74: 3418-3422.
- [188] Wang J, Smerdon SJ, Jager J, Kohlstaedt LA, Rice PA, Friedman JM, Steitz TA. (1994). *Proceedings of the National Academy of Sciences USA*. 91: 7242-7246.
- [189] Wang JY, Ling H, Yang W, Craigie R. (2001). *EMBO Journal*. 20: 7333-7343.
- [190] Wang S-W, Noonan K, Aldovini A. (2004). *Journal of Virology*. 78: 716-723.
- [191] Weber IT, Miller M, Jaskolski M, Leis J, Skalka AM, Wlodawer A. (1989). *Science*. 243: 928-931.
- [192] Wieggers K, Rutter G, Kottler H, Tessmer U, Hohenberg H, Krausslich HG. (1998). *Journal of Virology*. 72: 2846-2854.
- [193] Wills JW, Craven RC. (1991). *AIDS*. 5: 639-654.
- [194] Wlodawer A, Miller M, Jaskolski M, Sathyanarayana BK, Baldwin E, Weber IT, Selk LM, Clawson L, Schneider J, Kent SB. (1989). *Science*. 245: 616-621.
- [195] Wondrak EM, Nashed NT, Haber MT, Jerina DM, Louis JM. (1996). *Journal of Biological Chemistry*. 271: 4477-4481.
- [196] Wu X, Liu H, Xiao H, Conway JA, Hunter E, Kappes JC. (1997). *EMBO Journal*. 16: 5113-5122.
- [197] Wu X, Liu H, Xiao H, Kim J, Sesaiah P, Natsoulis G, Boeke J, Hahn B, Kappes J. (1995). *Journal of Virology*. 69: 3389-3398.
- [198] Yu X, Yuan X, Matsuda Z, Lee TH, Essex M. (1992). *Journal of Virology*. 66: 4966-4971.
- [199] Yu XF, Dawson L, Tian CJ, Flexner C, Dettenhofer M. (1998). *Journal of Virology*. 72: 3412-3417.
- [200] Yu XF, Matsuda Z, Yu QC, Lee TH, Essex M. (1995). *Journal of General Virology*. 76: 3171-3179.
- [201] Zheng R, Jenkins TM, Craigie R. (1996). *Proceedings of the National Academy of Sciences USA*. 93: 13659-13664.
- [202] Zheng YH, Plemenitas A, Linnemann T, Fackler OT, Peterlin BM. (2001). *Current Biology*. 11: 875-879.
- [203] Zhou W, Parent LJ, Wills JW, Resh MD. (1994). *Journal of Virology*. 68: 2556-2569.
- [204] Zybarth G, Krausslich HG, Partin K, Carter C. (1994). *Journal of Virology*. 68: 240-250.