CHAPTER 3

Molecular Biology of HIV

B. MATIJA PETERLIN

I. INTRODUCTION

This chapter deals with the molecular biology of human and simian immunodeficiency viruses (HIV-1, HIV-2, SIV) (Myers *et al.*, 1993; see also Fultz, 1994; Chapter 1, this volume). They belong to the lentivirus genus of the Retroviridae family and cause acquired immunodeficiency syndrome (AIDS) in humans and monkeys (Coffin, 1992; Gardner *et al.*, 1994). Since their genomes contain many open reading frames (ORFs) and since they evolved complex regulatory pathways, they are also called complex retroviruses [as contrasted with simple retroviruses like murine leukemia virus (MuLV)] (Cullen, 1991; Luciw and Leung, 1992). Most data are presented on HIV-1, which is simply called HIV. However, when necessary, examples from HIV-2 and SIV are included. The chapter starts out with the provirus integrated into the host genome, where the provirus behaves like a regular eukaryotic gene. The chapter follows viral transcription, the creation of new virions, their attachment and entry into $CD4^+$ cells, and concludes with reverse transcription and integration of the provirus into the cellular DNA. Along this journey, structures and functions of viral proteins are highlighted.

II. HIV PROVIRUS

Integrated into the host genome, HIV appears to be like a human gene, measuring between 9 and 10 kb (Fig. 1) (Myers *et al.*, 1993). At the 5′ and 3′ ends of the gene are the long terminal repeats (LTRs). They contain untranslated 3′ [U3: 400 base pairs (bp)], repeated (R:100 bp), and untranslated 5′ (U5: 100 bp) sequences (Myers

B. MATIJA PETERLIN • Howard Hughes Medical Institute, Departments of Medicine, Microbiology, and Immunology, University of California School of Medicine, San Francisco, California 94143-0724.

J. A. Levy (ed.), *The Retroviridae*

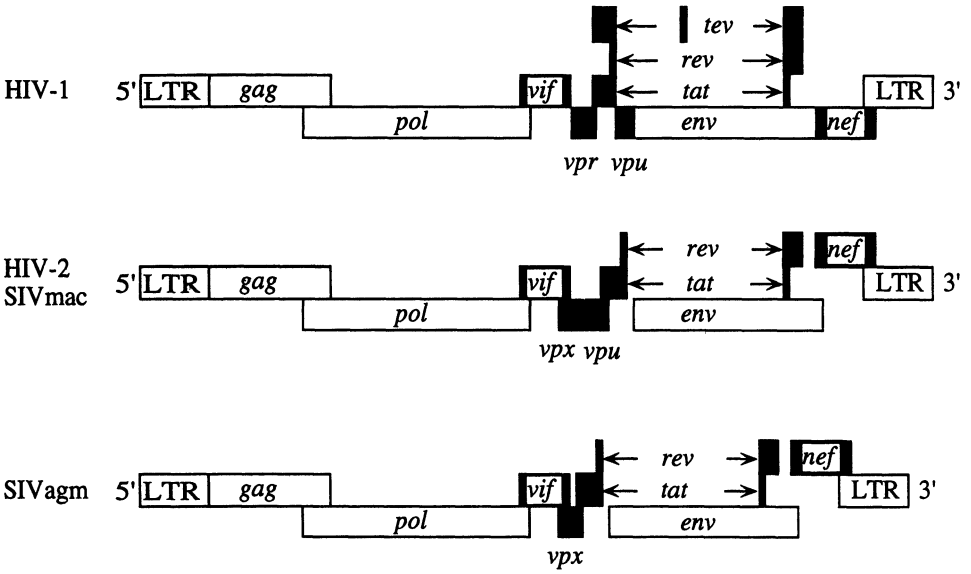


FIGURE 1. Genomic organization of HIV-1, HIV-2/SIV_{mac}, and SIV_{agm}. All three primate lentiviruses have similar genomic organization and length (between 9 and 10 kb). Structural genes are represented by open squares; regulatory and accessory genes are represented by black squares. *Vpr* is found in HIV-1, *vpx* is found in HIV-1 and SIV. *Vpu* is present in HIV-1, HIV-2, and SIV_{mac}, but not in SIV_{agm}. Both 5′ and 3′ LTRs are diagrammed and they flank the viral genome. HIV-1 also codes for *tev*, which contains *tat*, *rev*, and part of *env* sequences.

et al., 1993). Transcription starts at the 5′ U3/R junction and ends at the 3′ R/U5 junction. Full-length transcripts are capped at the 5′ end with 7-methylguanosine and are polyadenylated at the 3′ end after the AAUAAA and downstream GU-rich sequences by cellular enzymes (Haseltine, 1991; Vaishnav and Wong-Staal, 1991; Clements and Wong-Staal, 1992; Luciw and Shacklett, 1993). Poly A tails measure 150 nucleotides (nt). Capping, splicing, and polyadenylation further stabilize viral transcripts in the nucleus (DeZazzo *et al.*, 1992; Malim and Cullen, 1993). The 5′ Poly A is occluded or not accessible to the polyadenylation machinery most likely because it is very near to the cap site, it is relatively inefficient and requires signals in U3, or both (Cherrington and Ganem, 1992; Imperiale and DeZazzo, 1991; Weichs an der Glon *et al.*, 1991). Likewise, the 3′ LTR is occluded or its DNA is unable to bind regulatory proteins due to read-through transcription, and thus functions poorly as a promoter element (Eggermont and Proudfoot, 1993; Luciw and Leung, 1992).

HIV contains nine ORFs (Fig. 1 and Table I) (Luciw and Shacklett, 1993; Myers *et al.*, 1992). From the 5′ direction, they are *gag*, *pol*, *vif*, *vpr*, *tat*, *rev*, *vpu*, *env*, and *nef* (Haseltine, 1991; Vaishnav and Wong-Staal, 1992; Clements and Wong-Staal, 1992; Luciw and Shacklett, 1993; Myers *et al.*, 1993). Of these, *gag* and *env* code for multiple structural proteins, which shape the budding virions and promote viral entry into cells. *Pol* directs the synthesis of protease (PR), reverse transcriptase (RT), and integrase (IN). Other ORFs code for single proteins that have stronger (Nef, Tat, Rev) and weaker (Vif, Vpr, Vpu) regulatory roles (Table I). To direct the synthesis of all these proteins, the virus uses several strategies.

TABLE I. Genes and Proteins of Primate Lentiviruses[a]

Gene	TCCL[b]	Protein size (kDa)	Subunit size (kDa)	Abbreviation	Name	Function
gag	Yes	pr55^{gag}		Gag	Group-specific antigen	Virion assembly
			p17	MA (matrix)		Virion structure
			p24	CA (capsid)		Nuclear entry of preintegration complex
			p9, p7	NC (nucleocapsid)		
pol	Yes	pr160$^{gag\text{-}pol}$	p10	PR (protease)	Protease	Processing of Gag
			p51/66	RT (reverse transcriptase/ RNase H)	RNA-dependent DNA-polymerase; Ribonuclease H	Copies viral RNA into DNA Removes RNA
			p32	IN (integrase)	Integrase	Proviral integration
vif	No[b]	p23		Vif	Virion infectivity factor	Virion uncoating, other?
vpx[c]	No	p16		Vpx	Viral protein X	Nuclear entry of preintegration complex
vpr	No	p15		Vpr	Viral protein R	
tat	Yes	p14		Tat	Transactivator	Binds TAR, increases rates of transcription
rev	Yes	p19		Rev	Regulator of expression of virion proteins	Binds RRE, inhibits splicing, increases cytoplasmic transport of viral RNA
vpu[d]	No	p16		Vpu	Viral protein U	Virion release, increases degradation of CD4
env	Yes	gp160		Env	Envelope	Virion receptor
			gp120/130	SU (surface)		
			gp41/32	TM (transmembrane)		
nef	No	p27		Nef	Negative factor	Removes CD4 from cell surface, affects signaling

[a]Given are names of viral genes and proteins, their sizes both as precursor and processed proteins, abbreviated names and functions, including whether they are needed for replication of virions in tissue culture cells. The nomenclature is according to Gallo *et al.* (1988). Genes encoded by HIV-1, HIV-2, and SIV are provided. By convention, genes are italicized and protein products are not. In addition, small p in front of a number denotes protein, gp, glycoprotein, and pr, precursor protein.
[b]Needed to grow in tissue culture cell lines (TCCL); growth without *vif* also depends on cell type.
[c]Encoded only by HIV-2 and SIV.
[d]Encoded only by HIV-1 and SIV_{cpz}.

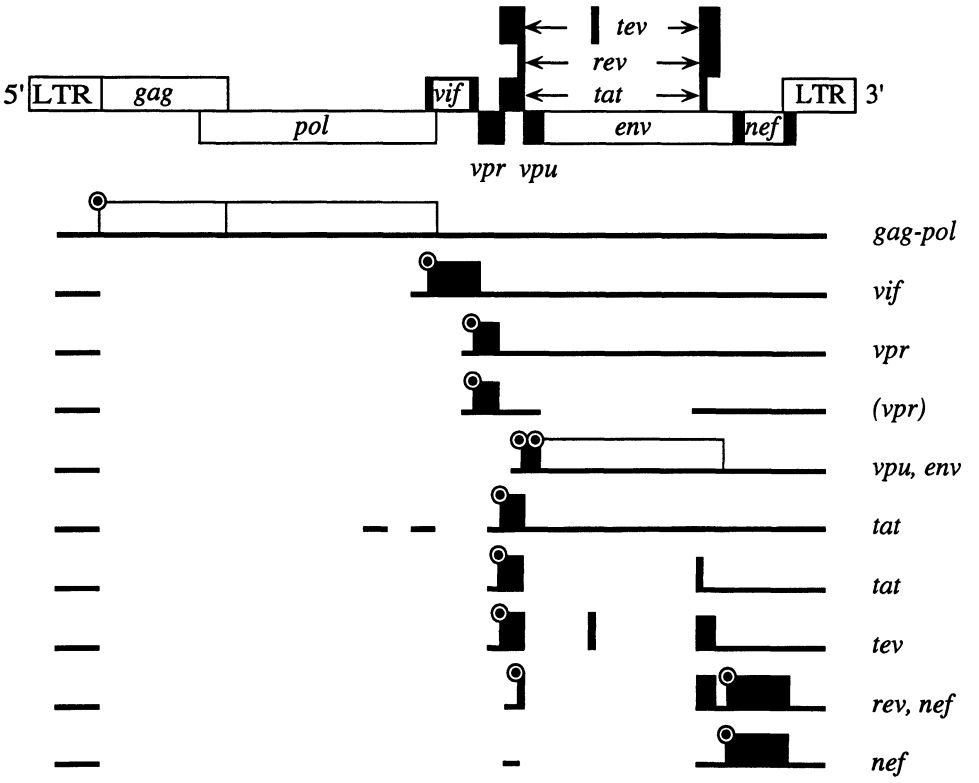


FIGURE 2. Splicing patterns of HIV-1 transcripts (Pavlakis *et al.*, 1992). At least ten qualitatively different transcripts are expressed from the HIV-1 genome. They are represented from the least to most spliced variants from top to bottom. Thus, the longest transcripts represent unspliced or genomic RNA, which codes for the Gag-Pol precursor and is packaged into new virions. Moreover, this RNA leads to translational frameshifting, which expresses the Pol precursor. Two different singly spliced transcripts code for Vif, Vpr, Vpu, and Env. These RNA species depend on Rev for transport from the nucleus to the cytoplasm and are called late transcripts. Multiply spliced transcripts code for Vpr, Tat, Tev, Rev, and Nef. These RNA species are translocated independently of Rev and are called early transcripts. At least three different transcripts code for proteins with Tat activity and they are all multiply spliced. Only two transcripts are differentially translated and have different ATG start codons. They code for Vpu and Env and Rev and Nef. Small dark concentric circles indicate translational start sites. Open and black squares represent ORFs.

First, several different HIV transcripts are expressed (Fig. 2) (Pavlakis *et al.*, 1992). Early after viral transcription, multiply spliced RNA species appear in the cytoplasm (Cullen and Greene, 1989; Kim *et al.*, 1989; Pomerantz *et al.*, 1990; Haseltine, 1991). They code for Nef, Tat, Tev, and Rev. Next, singly spliced transcripts appear. They code for Vif, Vpr, Vpu, and Env. Finally, full-length, genomic transcripts are observed. They code for Gag and Pol. Vif, Vpr, Vpu, Env, Gag, and Pol are expressed late in the viral replicative cycle (Cullen and Greene, 1989; Kim *et al.*, 1989; Pomerantz *et al.*, 1990; Haseltine, 1991). Differential splicing of HIV transcripts is due to inefficient splicing signals on the RNA, the use of alternative splicing pathways, and the viral Rev protein (Pavlakis *et al.*, 1992).

Second, Gag and Pol are synthesized from the same transcripts by a mechanism called translational frame shifting (Fig. 3) (Jacks *et al.*, 1988; Jacks, 1990). Requirements are an RNA sequence of about seven nucleotides (nt) (UUUUUUA in HIV-1), followed by an RNA stem-loop. The base at the frame shifting site is translated once in the 0 frame and once in the −1 frame. Most likely, the transfer RNA (tRNA) slips on the ribosomal acceptor site because of the retarded movement of polysomes due to the RNA stem-loop (Chamorro *et al.*, 1992; Vickers and Ecker, 1992). The ratio of synthesized Gag to Pol proteins is 20:1 (Hatfield *et al.*, 1992).

Third, Gag, Pol, and Env are cleaved into subunit proteins (Fig. 4) (Luciw and Shacklett, 1993). Gag-Pol precursor, pr160, is digested by the viral protease into four Gag proteins [p17 (MA, matrix); p24 (CA, capsid); p7 (proline-rich); and p9 (NC, nucleic acid binding)] and PR (p10), RT (p66, p51), and IN (p32) (Henderson *et al.*, 1992). Env precursor (gp160) is processed by cellular proteases into gp120 (SU, surface) and gp41 (TM, transmembrane) (Hunter and Swanstrom, 1990). Thus from a simple 9-kb gene, 16 different proteins are synthesized (Fig. 4). They are involved in every aspect of the viral replicative cycle. As such, they are also attractive targets for antiretroviral therapy.

A. Transcription

The 5′ LTR serves as the site of initiation of viral transcription (Fig. 5) (Nabel, 1993; Jones and Peterlin, 1994; see Chapter 2, this volume). It is composed of several *cis*-acting elements to which *trans*-acting factors bind. In the 5′ U3 region, there are binding sites for the activator protein-1 (AP-1/COUP), c-Myb, and nuclear factor of activated T cells (NF-AT). Distal enhancer sequences from positions −105 to −80 bind the upstream stimulatory factor-1 (USF-1), chicken Ets oncogene (Ets-1), and lymphocyte enhancer factor (LEF). Proximal enhancer sequences from positions −104 to −80 bind nuclear factor kappa B (NF-κB) (Nabel, 1993). Promoter elements from positions −79 to −1 contain Sp1, TATA, and initiator sequences. The 3′ sequences from positions +1 to +60 contain initiator of short transcript (IST), and *trans*-activation response (TAR) elements and bind leader binding protein-1/untranslated region binding protein-1 (LBP-1/UBP-1), T-cell factor-1/untranslated region binding protein-2 (TCF1/UBP-2), and CAAT transcription factor/nuclear factor-1 (CTF/NF-1) (Nabel, 1993; Jones and Peterlin, 1994). IST promotes the formation of prematurely terminated, nonpolyadenylated transcripts (Sheldon *et al.*, 1993), and TAR binds Tat as an RNA stem-loop (Peterlin *et al.*, 1993; see Chapter 2, this volume).

Distal and proximal enhancer elements are involved in activated transcription from the LTR. Not only are LEF and Ets-1 enriched in T cells, but NF-AT and NF-κB translocate from the cytoplasm to the nucleus after T-cell activation (Nabel, 1993). Moreover, NF-κB is a potent mitogen-induced transcriptional activator. They render the LTR responsive to the physiological state of the infected cell and greatly increase rates of initiation of viral transcription. The 5′ promoter elements determine the site of initiation of HIV transcription. Sp1, TATA, and initiator elements also attract basal transcription factors to the LTR via their coactivators, TATA-binding protein (TBP)-binding factors (TAFs) and TFIII/HIP (HIV initiator protein) (Jones and Peterlin, 1994).

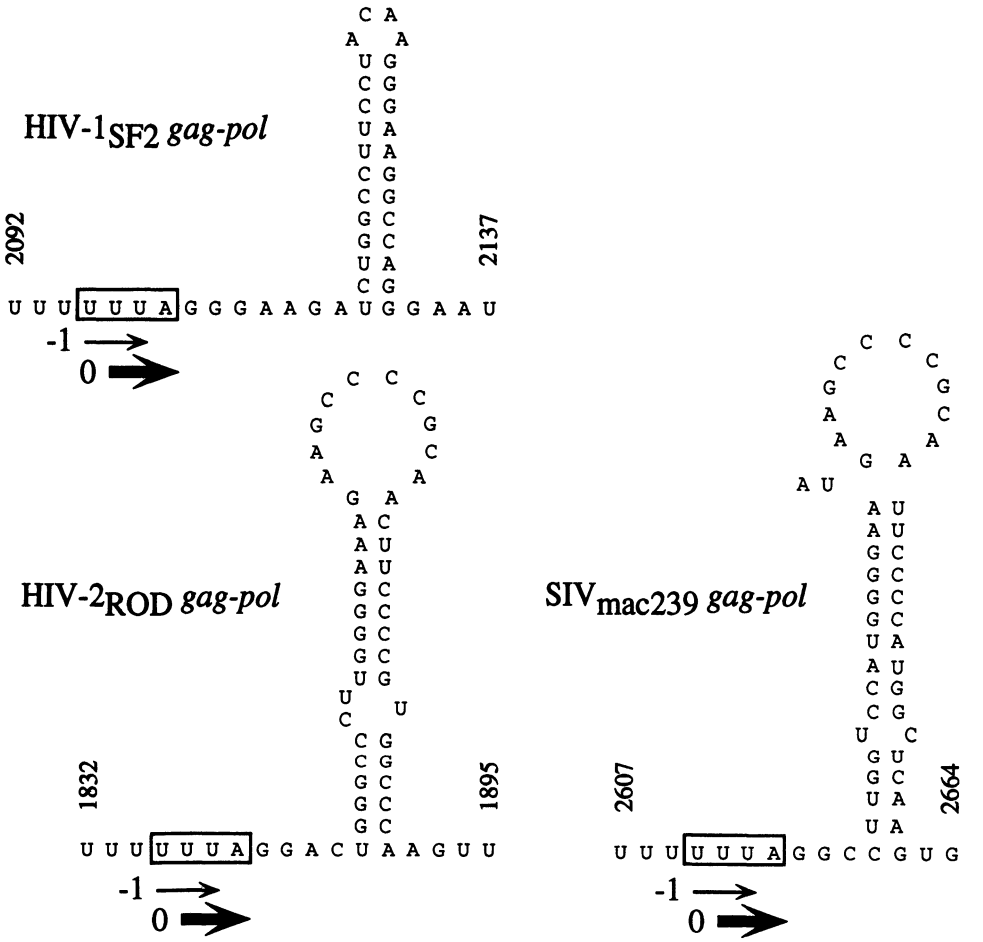


FIGURE 3. Translational frameshifting in HIV-1, HIV-2_{rod}, and SIV_{mac239}. The *pol* gene is translated only in about 5% of genomic viral transcripts. This process occurs because the *gag* and *pol* genes are not in the same reading frame. Thus, ribosomal frameshifting has to occur for the translation of *pol*. The conserved sequences at the junction of *gag* and *pol* are involved in this frameshifting. In the simplest model, which is called the simultaneous slippage model, the "shifting" codon (represented by boxed sequences) and UUUA are translated both in the zero frame and in the −1 frame. In the zero frame (thick arrow), this yields the UUA codon, and in the −1 frame (thin arrow), this yields the UUU codon. The slippage occurs because the RNA stem-loop, which is located 3′ to the shifting sequence, retards the ribosomal movement along the transcript. Because of the U-rich content of the shifting codon, this process results in the slippage of the reading frame. All lentiviruses code for a single −1 frame shift at the junction between *gag* and *pol* genes. Note that sequences and structures of the RNA stem-loops differ among these lentiviruses. Positions in the *env* gene are indicated by numbers. (Modified from Jacks, 1990.)

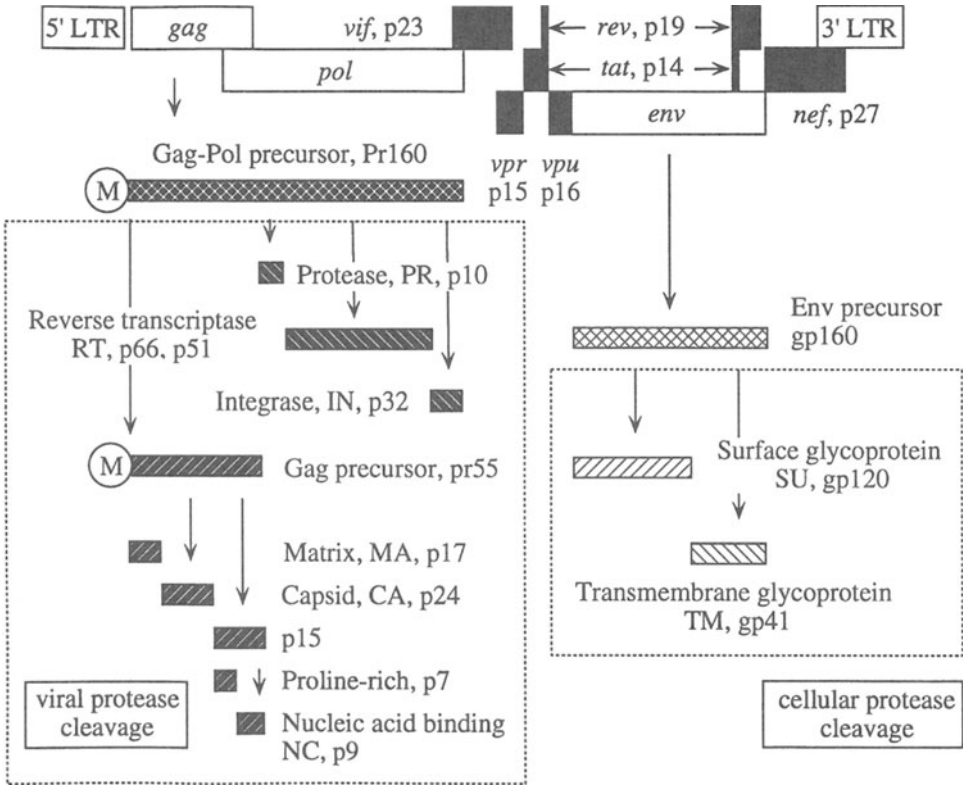


FIGURE 4. Processing of viral proteins. Proteins translated from ten different viral transcripts are processed in a complex fashion. From nine proteins, which include Tev (not diagrammed), are created 16 proteins that form the virion structure and determine viral enzymatic regulatory and accessory functions. The Gag-Pol precursor of 160 kDa ($pr160^{gag\text{-}pol}$) is processed by the viral protease, which is an aspartyl protease, into seven proteins including Gag proteins (p17, matrix; p24, capsid; p7, proline-rich; and p9, nucleic acid binding) and p10 (protease), p66, p55 (RT, reverse transcriptase), p32 (integrase). The Env precursor which is a glycoprotein of 160 kDa (gp160), is processed by cellular proteases into the surface glycoprotein of 120 kDa (gp120) and transmembrane glycoprotein of 41 kDa (gp41). Viral regulatory and accessory proteins, which include Tat, Tev, Rev, Nef, Vif, Vpr, and Vpu, are not processed further.

Promoter elements in the 3′ portion of the 5′ LTR generate short viral transcripts and form the TAR RNA stem-loop (Sheldon *et al.*, 1993; Peterlin *et al.*, 1993; Jones and Peterlin, 1994). The viral *trans*-activator Tat binds to TAR to increase greatly contributions of all other DNA-binding transcriptional activators (Frankel, 1992a; Jones and Peterlin 1994; Peterlin *et al.*, 1993). Very high levels of viral transcription and replication result from these protein–nucleic acid interactions. Finally, several other viral activators, for example, those of DNA viruses such as herpes simplex virus (HSV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV), and those of retroviruses such as human T-cell leukemia virus-1 (HTLV-1) and human foamy virus (HFV) have been demonstrated to increase rates of transcription

from the LTR by interacting with different enhancer and promoter elements (Fig. 5) (Nabel, 1993; Jones and Peterlin, 1994; see Chapters 2 and 4, this volume).

III. REGULATORY PROTEINS

HIV codes for three major regulatory proteins, Nef, Tat, and Rev (Figs. 1, 2, 4, and 6) (Cullen and Greene, 1989; Karn, 1991; Rosen and Dillon, 1991; Cullen, 1992; Felber and Pavlakis, 1993; Luciw and Shacklett, 1993). They are expressed from multiply spliced viral transcripts that appear early after transcriptional activation of the provirus (Fig. 2). None of their protein products are packaged into virions (see Section IX, Fig. 18). As small proteins, they diffuse easily between the cytoplasm and the nucleus. They are essential for high levels of viremia, viral cytopathology, and progression of disease. Their mechanisms of action are only now becoming better understood (Cullen and Greene, 1989; Rosen and Dillon, 1991; Cullen, 1992; Karn, 1991; Felber and Pavlakis, 1993; Luciw and Shacklett, 1993).

A. Nef

Nef is the earliest expressed viral protein (Robert-Guroff *et al.*, 1990). It contains 210 to 250 amino acids and measures 25 to 30 kDa (Myers *et al.*, 1993; Greenway *et al.*, 1994). In HIV-1 and SIV_{CPZ}, the translation of Nef initiates at the 3′ end of the *env* gene and extends into U3 sequences (Fig. 1) (Myers *et al.*, 1993). In HIV-2 and other SIV strains, the 5′ and 3′ ends of *nef* and *env* overlap; however, *nef* is in a different translation frame (Fig. 1) (Myers *et al.*, 1993). Two different multiply spliced viral transcripts code for Nef. Whereas one of these codes for Nef only, the other codes for Rev and Nef (Fig. 2). Moreover, most *nef* transcripts contain two initiation codons at residues 1 and 20, and both are used (Greenway *et al.*, 1994). When the first methionine is used, Nef is posttranscriptionally modified by myristylation and phosphorylation (Guy *et al.*, 1990; Samuel *et al.*, 1991). The initiator methionine is removed and myristic acid is N-linked to the glycine at position 2 by cellular enzymes (Kan *et al.*, 1986). The N-terminal myristylation targets Nef to cellular and plasma membranes and is important for its function (Yu and Felsted, 1992). Of questionable importance is its phosphorylation on a serine or threonine near the N-terminus (Samuel *et al.*, 1991).

No crystallographic or nuclear magnetic resonance (NMR) structures of Nef exist. However, conserved among all Nef proteins is an N-terminal acidic region rich in glutamic acid residues, which is followed by Pro-Xaa, Xaa repeats. Next, Gly^{130}-Pro^{131}-Gly^{132}-Ile^{133}/Val^{133} are predicted to form a β-turn. A motif of repeated leucines and another acidic region are found near the C-terminus (Shugars *et al.*, 1993). Most Nef proteins also contain several cysteines, which form intermolecular disulfide bonds, so that Nef exists as a homodimer in cells (Kienzle *et al.*, 1993). Furthermore, the sequence of Nef contains similarities to Ras and Src kinases, yeast DNA-binding protein RAP-1, scorpion neurotoxin, class II human leukocyte antigen (HLA), and mammalian G proteins (Guy *et al.*, 1987; Shugars *et al.*, 1993). In reference to the last point, early GTP binding and autophosphorylation studies by Nef have not been validated (Guy *et al.*, 1987).

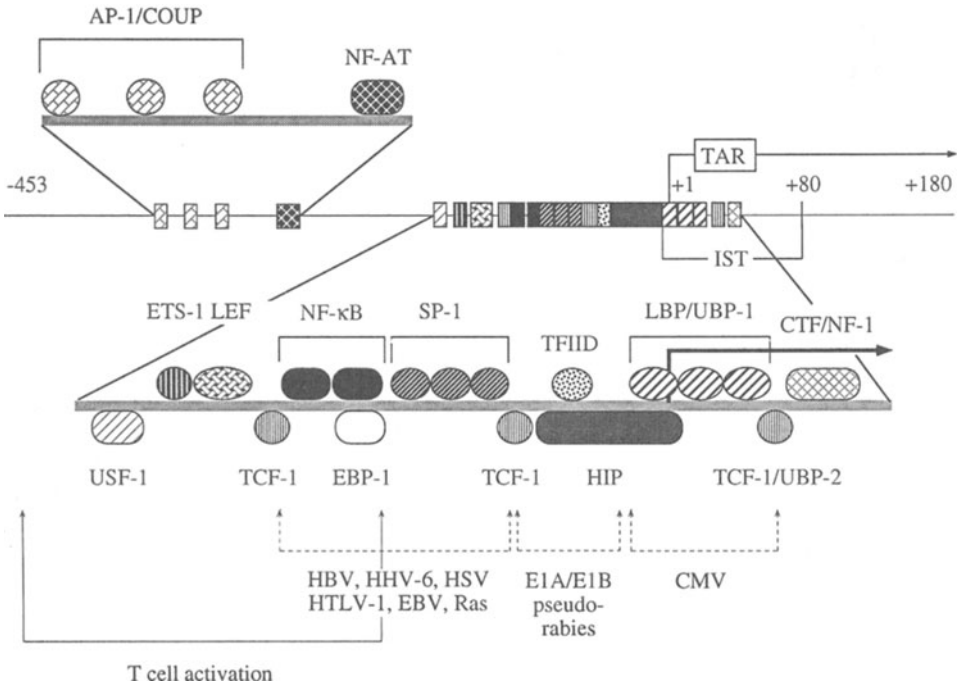


FIGURE 5. The HIV-1 LTR. The HIV-1 LTR measures 633 nucleotides and contains binding sites for many different DNA-binding proteins. From the 5′ to 3′ direction they are AP-1/coup (activator protein-1), NF-AT (nuclear factor-activated T cells), USF-1 (upstream stimulatory factor-1), Ets-1 (chicken Ets oncogene), LEF (lymphocyte enhancer factor), TCF-1 (T-cell factor-1), NF-κB (nuclear factor of kappa B), EBP-1 (enhancer binding protein-1), SP-1, TFIID (transcription factor IID), HIP (HIV initiator protein), LBP/UBP-1 (leader binding protein/untranslated region binding protein-1), UBP-2 (untranslated region binding protein-2), and CTF/NF-1 (CAAT transcription factor/nuclear factor-1). Of these proteins NF-AT and NF-κB are activated following T-cell activation, Ets-1 and LEF are lymphoid cell specific, and SP-1, TFIID, and LBP-1 are important for basal promoter function and the positioning of RNA polymerase II. Activities of other proteins, for example, AP-1, USF-1, TCF-1, and HIP, have not been determined. Under the diagram of the LTR are given regions that respond to different stimuli. For example, T-cell activation targets NF-AT and NF-κB sequences, whereas other viral *trans*-activators such as the X protein of HBV (hepatitis B virus) and activators of HHV6 (human herpes virus 6), HSV (herpes simplex virus), HTLV-1 (human T cell leukemia virus), EBV (Epstein-Barr virus), and Ras proto-oncogene target NF-κB and Sp1 sequences. E1A, E1B, and pseudo-rabies early activators activate via the TATA box and the CMV early activator targets LBP/UBP-1 sites. The TAR (*trans*-activation response region) element is diagrammed after the site of initiation of HIV-1 transcription on the RNA. IST is the DNA element that overlaps TAR. Other potential DNA-binding sites and DNase I hypersensitive sites in the LTR have been described (Jones and Peterlin, 1994).

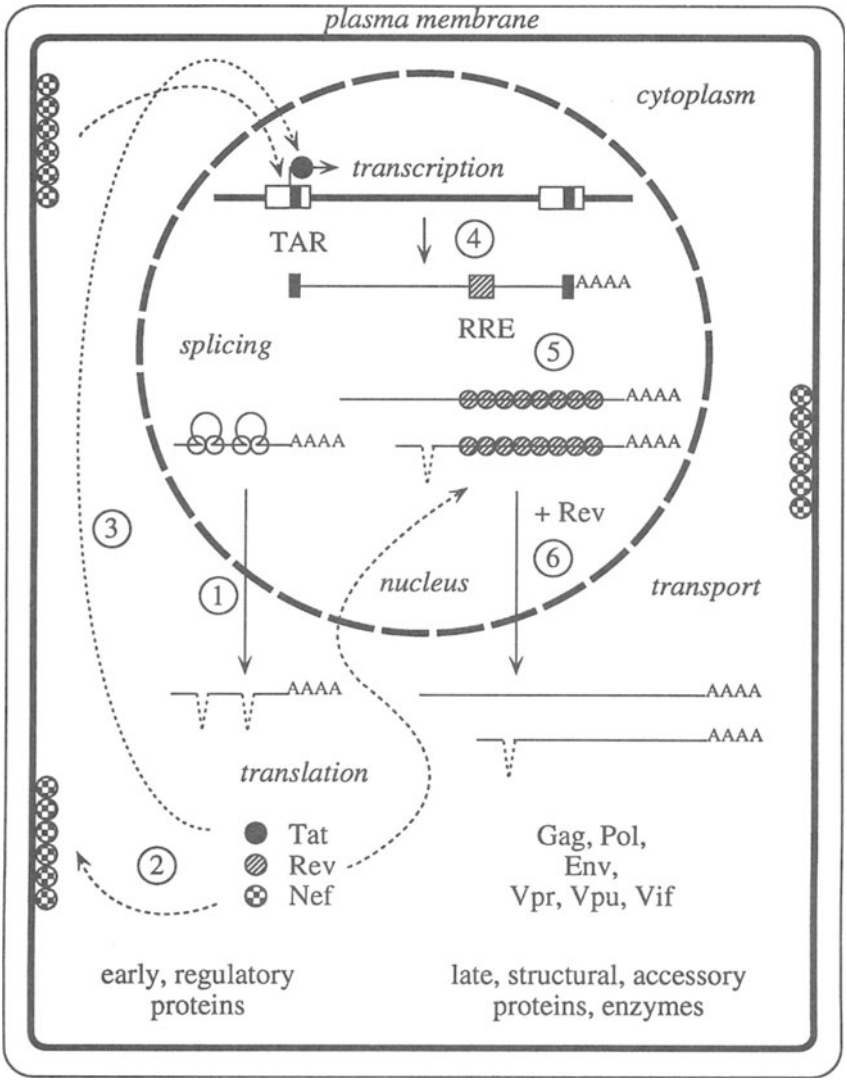


FIGURE 6. Viral regulatory proteins and their mechanisms of action. (1) Nef, Tat, and Rev are the earliest viral proteins expressed and they have profound effects on the rates of viral replication. Of these, Nef is a myristylated protein that is associated with the cellular membranes and the plasma membrane and leads to greatly increased levels of viral replication in CD4-positive lymphocytes and to viremia in monkeys. (2) This event can occur via the activation of cellular signaling pathways and translocation of NF-κB from the cytoplasm to the nucleus. (3) Tat then interacts with TAR to potentiate rates of viral transcription. (4) Finally, Rev interacts with the RRE (5) to transport singly-spliced and unspliced viral RNA species from the nucleus to the cytoplasm, where viral structural and accessory proteins and enzymes are synthesized so that virion budding and release can proceed. This completes the late phase of viral replication. In this scheme, all regulatory proteins play positive roles such that Nef interacts with cellular activation pathways to increase viral transcription in synergy with Tat and Rev and increases the availability of genomic RNA for viral packaging. All of these regulatory proteins are expressed early and are translated from multiply spliced viral transcripts.

Nef decreases the expression of CD4 on T cells (Garcia and Miller, 1991; Aiken *et al.*, 1994). As such, it joins gp120 and Vpu, which have similar effects (Capon and Ward, 1991; Willey *et al.*, 1994). The target for Nef is the double Leu^{413}-Leu^{414} motif in the intracytoplasmic domain of CD4 (Aiken *et al.*, 1994). Although these effects of Nef have been observed also in nonlymphoid cells expressing CD4, Nef does not bind to CD4 directly, nor does it affect its biosynthesis. Recently, Nef was demonstrated to increase the rate of endocytosis of CD4 on the cell surface, which could result from effects of Nef on the physiology of the infected cell (Aiken *et al.*, 1994). This removal of CD4 from the cell surface could prevent the reinfection of cells that already harbor the provirus (Karn, 1991).

Nef has also profound effects on the infection by SIV in monkeys (Fig. 7) (Kestler *et al.*, 1991). SIV strains, which express truncated Nef proteins, do not lead to viremia and death in Rhesus macaques. Moreover, the premature stop codon in *nef*, which exists in SIV_{mac239}, reverts to the ORF, so that the synthesis of full-length Nef is observed. This leads to high levels of viremia and progression of disease (Kestler *et al.*, 1991). Thus, Nef is not only essential for high levels of SIV replication *in vivo*, but viral strains lacking the *nef* gene are candidates for the attenuated vaccine against SIV infection (Desrosiers, 1992). This positive effect of Nef has also been observed in infected peripheral blood $CD4^+$ lymphocytes and is especially pronounced at low multiplicity of infection, where HIV strains with Nef grow better (Chowers *et al.*, 1994; Miller *et al.*, 1994; Spina *et al.*, 1994).

In sharp contrast, Nef behaves like a negative factor or has no effects in human T-cell lines (Fig. 7) (Cheng-Mayer *et al.*, 1989; Niederman *et al.*, 1989, 1991; Garcia *et al.*, 1993). These cells favor truncated Nef proteins. However, when they express the full-length protein, Nef inhibits HIV-1 transcription and blocks the activation of NF-κB, AP-1, and IL-2 (Niederman *et al.*, 1989, 1991; Luria *et al.*, 1991). To reconcile these differences, intracytoplasmic Nef has correlated with the inhibition and its expression on the cell surface has correlated with the activation of signaling from the T-cell antigen receptor (Baur *et al.*, 1994). In these studies, Nef also associated with two proteins of 72 and 62 kDa, which were phosphorylated on a serine residue (Sawai *et al.*, 1994). One of these two proteins could be a serine/threonine kinase.

Thus, Nef might function like a signaling molecule and perturb the physiology of the infected cell. In $CD4^+$ lymphocytes and in the host, these effects are positive and lead to cellular activation and increased virus expression from the LTR. In immature T cells, the same signals lead to the cessation of cell growth and apoptosis. Thus, it is not surprising that only viral strains, which contain open *nef* genes, cause thymic involution and loss of early T cells in the SCID/hu mouse model of HIV infection (Bonyhadi *et al.*, 1993; Lindemann *et al.*, 1994). The complexity of this regulation by Nef is increased by its use of two different start codons, only one of which leads to the synthesis of a protein that can be myristylated and phosphorylated (Greenway *et al.*, 1994).

B. Tat

Tat is a protein of 86 to 101 amino acids that measures 14 to 15 kDa (Fig. 8) (Cullen, 1992; Frankel, 1992a; Karn and Graeble, 1992; Peterlin *et al.*, 1993; Jones and Peterlin, 1994). It is translated from multiply spliced viral transcripts (Fig. 2). Its

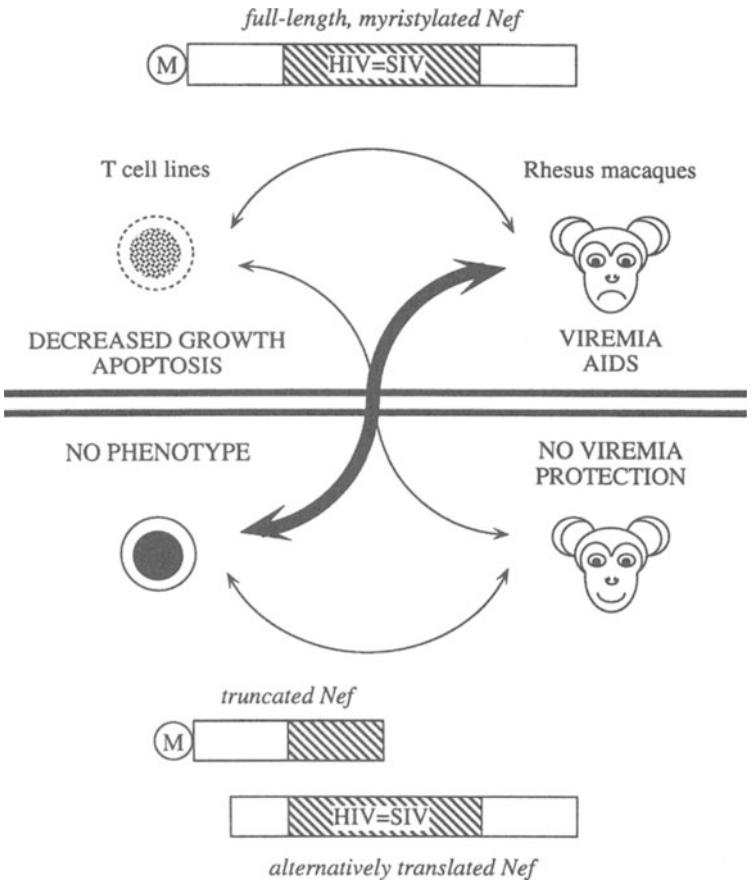


FIGURE 7. The paradox of Nef. Nef of primate lentiviruses is an abundant myristylated protein of 25 to 32 kDa. When the full-length Nef is expressed in SIV_{mac239}, it leads to viremia and death from AIDS in infected monkeys, but deletions of Nef result in low to no viremia and protection against a subsequent viral challenge. In sharp contrast, truncated or mutant Nef proteins are selected in human T-cell lines. In these cells, full-length Nef leads to decreased rates of growth and apoptosis. The domains that are conserved among the HIV and SIV Nef proteins are diagrammed as squares with slanted lines and the forms of Nef that are preferentially expressed in T-cell lines and monkeys are depicted on top and bottom of the figure, respectively. Additionally, some viral Nef proteins are translated from the second initiator methionine, which leads to a smaller Nef that is neither myristylated nor phosphorylated.

coding exons flank the *env* gene and the first and invariant exon codes for 72 amino acids, which represent the functional domain of Tat. The second exon is largely dispensable for the function of Tat of HIV-1, but is important for RNA binding of Tat of HIV-2 (Tong-Starksen *et al.*, 1993). In fact, only the first 57 amino acids of Tat are required for efficient *trans*-activation. Tat is localized in the nucleus via the nuclear localization signal Gly^{48}-Arg^{49}-Lys^{50}-Lys^{51}-Arg^{52} in its basic domain (Hauber *et al.*, 1989). There, Tat binds to an RNA structure called the *trans*-activation response

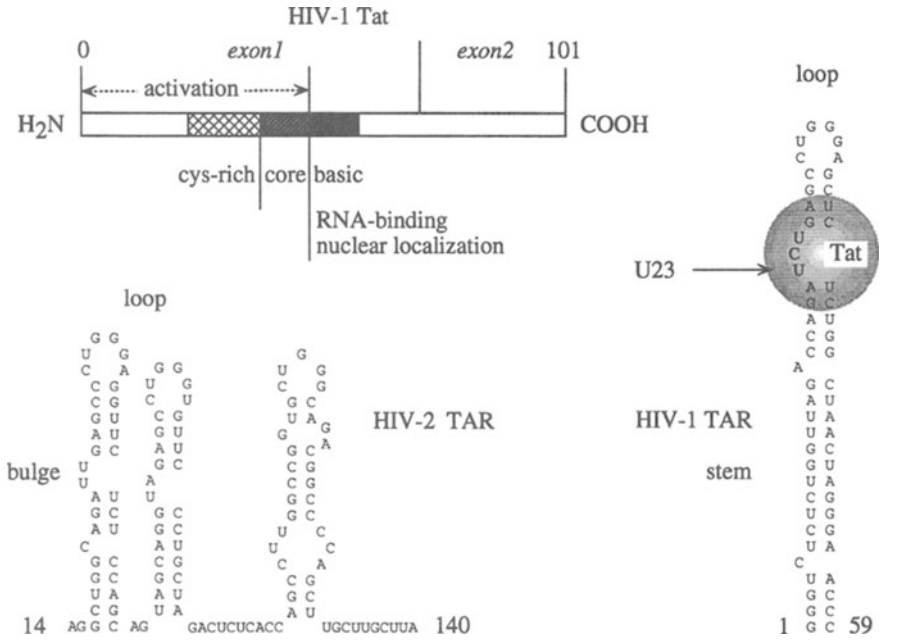


FIGURE 8. The structure of Tat. (Top) Tat of HIV-1_{SF2} contains 101 aa and is translated from multiply-spliced viral transcripts of which exon 1 contains all Tat activity. In this region there are activation (N-terminal, cysteine-rich, and core) and basic (arginine-rich) domains. Most conserved among all lentiviral Tats are the cysteine-rich, core, and basic domains. (Right) Tat of HIV-1 binds to the 5′ bulge in TAR and the predominant nucleotides required for this binding are the lower U (U23) in the bulge and nucleotides just above and below the bulge. (Left) TAR of HIV-2 is more complex, containing three stem-loops where Tat of HIV-2 presumably binds to the bulges of both duplicated stem-loops. TARs of HIV-1 and HIV-2 have predicted free energy of −37 and −85 kcal, respectively (Jones and Peterlin, 1994).

(TAR) element (Rosen *et al.*, 1985). TAR is found at the 5′ end of all viral transcripts and forms an RNA stem-loop with a calculated free energy of −35 kcal (TAR of HIV-1) and −85 kcal (TAR of HIV-2/SIV) (Emerman *et al.*, 1987; Muesing *et al.*, 1987).

Tat can be divided into five functional domains: the N-terminal, cysteine-rich, core, basic, and C-terminal domains (Fig. 8) (Peterlin *et al.*, 1993; Jones and Peterlin, 1994). Of these, the core and basic domains are most conserved among Tat proteins of HIV-1, HIV-2/SIV, and other lentiviruses (Carroll *et al.*, 1991; Myers *et al.*, 1993). The N-terminal domain contains several acidic residues (Rappaport *et al.*, 1989). The cysteine-rich domain binds divalent cations and can form homodimers in solution (Frankel *et al.*, 1988). The core region is most invariant among all Tat proteins (Carroll *et al.*, 1991). The basic domain contains nine arginine and lysine residues, of which one arginine in the context of nine lysines is sufficient for the binding of Tat to TAR *in vitro* (Frankel, 1992b). Moreover, the basic domains from Rev and bacteriophage λ N protein can substitute for the basic domain of Tat and still lead to efficient *trans*-activation (Subramanian *et al.*, 1990). The C-terminal

domain of Tat is rich in glutamine and serine residues and can be phosphorylated (Peterlin *et al.*, 1993). However, this phosphorylation has no known function.

Although the structure of Tat proteins of HIV-1 and HIV-2/SIV have not been determined, the NMR structure of Tat of equine infectious anemia virus (EIAV) (Willbold *et al.*, 1994) and of a minimal lentiviral Tat (Mujeeb *et al.*, 1994), which contains the core and basic domains of Tats of EIAV and HIV-1, respectively, are known. Most of the Tat of EIAV is unstructured and compact, since both its N- and C-termini fold on the core domain. In the minimal lentiviral Tat, the basic domain forms an α-helix (Mujeeb *et al.*, 1994). The rest of the protein probably assumes a more stable conformation after binding specific cellular coactivators and RNA-tethering factors. Although only the basic domain is required for RNA binding *in vitro*, the entire 57 N-terminal amino acids of Tat are required for efficient Tat–TAR interactions *in vivo* (Luo *et al.*, 1993). Since the first 48 amino acids of Tat also represent its activation domain, sequences that activate transcription and tether Tat to TAR overlap. The removal of the basic domain also creates a *trans*-dominant negative form of Tat that decreases effects of Tat in cells (Modesti *et al.*, 1991).

Tat binds to TAR which forms a stable RNA structure (Fig. 8). TAR contains a 5′ bulge and a central loop on a short stem. Tat binds to the 5′ bulge and, in particular, to U23 (Fig. 9) (Dingwall *et al.*, 1990; Roy *et al.*, 1990; Frankel, 1992b). Nucleotides above and below the bulge are also required for this binding, namely G26/C39, A27/U38, A22/U40, and G21/C41 (Fig. 9) (Frankel, 1992b; Gait and Karn, 1993). Other cellular proteins bind to the loop in TAR. The best characterized of these is TRP-1 or TRP-185, which synergizes with Tat for *trans*-activation and may be required for efficient complex formation between Tat, TAR, and the transcription complex (Fig. 9) (Jones and Peterlin, 1994). TAR structures of HIV-1 and SIV-2 are longer (125 nt) and contain duplicated RNA stem-loops. Tat proteins of HIV-2 and SIV are also larger and contain 130 amino acids. There is some nonreciprocity between lentiviral Tat proteins and the TAR regions such that Tat of HIV-1 *trans*-activates efficiently TARs of HIV-1, HIV-2, and SIV, but Tat of HIV-2 functions inefficiently on TAR of HIV-1 (Emerman *et al.*, 1987; Jones and Peterlin, 1994). Recently, this observation has been explained by the less efficient binding of Tat of HIV-2 to a single TAR (Tong-Starksen *et al.*, 1993).

Tat is an essential viral protein that increases the number of all viral transcripts (Fig. 10). Although it increases the rate of viral transcription its precise mechanism of action is unknown (Cullen, 1992; Frankel, 1992a; Karn and Graeble, 1992; Peterlin *et al.*, 1993; Jones and Peterlin, 1994). Arguments for effects of Tat on rates of initiation and elongation by RNA polymerase II have been presented. Since TAR has to be synthesized for Tat to bind, the initial event has to include some transcription of the LTR. In fact, this early event can be followed by nuclear run-ons and levels of steady-state RNA, which reveal promoter proximal transcription and abundant prematurely terminated TAR transcripts in tissue culture cells and cells from infected humans (Kao *et al.*, 1987; Adams *et al.*, 1994). Since TAR DNA has been implicated in the formation of these short transcripts, these sequences in the LTR are also called inducer of short transcripts (IST) (Fig. 5) (Sheldon *et al.*, 1993). However, other experiments suggested that the TATA box alone assembles transcription complexes, which move along the DNA template to synthesize TAR (X. Lu *et al.*, 1993).

Can Tat modify these nonprocessive transcription complexes for further elon-

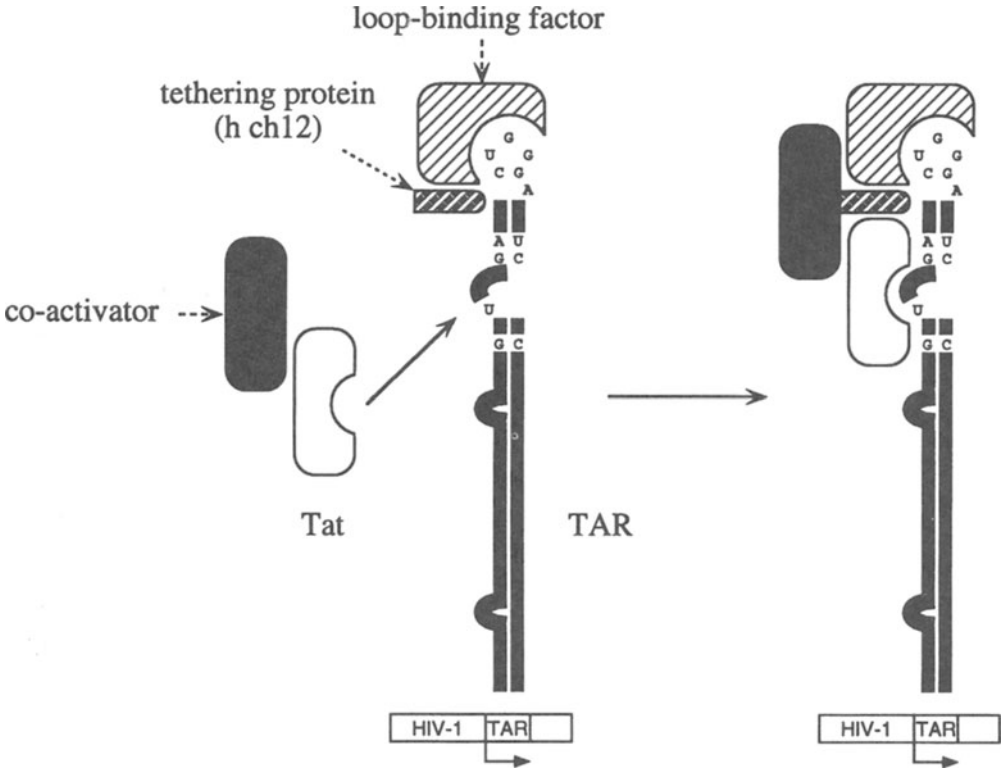


FIGURE 9. Interactions between Tat and TAR *in vivo*. From biochemical and genetic studies it is apparent that Tat of HIV-1 binds to the 5′ bulge in which U23 is the most important nucleotide. Nucleotides above and below the bulge are also required. All other nucleotides specify a stable stem structure and are exchangeable (Karn and Graeble, 1992; Frankel, 1992b). In addition, cellular loop-binding proteins bind to the loop in TAR and are required for optimal interactions between Tat and TAR in cells. However, no direct interactions between these loop-binding proteins and Tat have been demonstrated (Jones and Peterlin, 1994). In rodent cells genetic evidence suggest that a protein encoded on human chromosome 12 (h ch12) tethers Tat to these cellular loop-binding proteins so that a multiprotein complex leads to Tat *trans*-activation.

gation or does Tat reach back to initiate new processive transcription complexes? Most likely, Tat affects the assembly of transcription complexes, but at a later step (Fig. 10) (Jones and Peterlin, 1994). To this end, data from *in vitro* transcription systems suggest that Tat acts like transcription factor IIF (TFIIF) which associates with the RNA polymerase II following the formation of the preinitiation complex (Kato *et al.*, 1992). Posttranscriptional effects of Tat have also been described. For example, Tat has significant effects on translation of TAR containing RNA in the *Xenopus* oocyte (Braddock *et al.*, 1990).

To elucidate its mechanisms of action, several cellular proteins that interact with Tat have been described. First, a protein called Tat-binding protein-1 (TBP-1) was isolated by expression cloning (Nelbock *et al.*, 1990). It is a 46-kDa protein that decreases effects of Tat in cotransfected cells. Another protein of 32 kDa has been

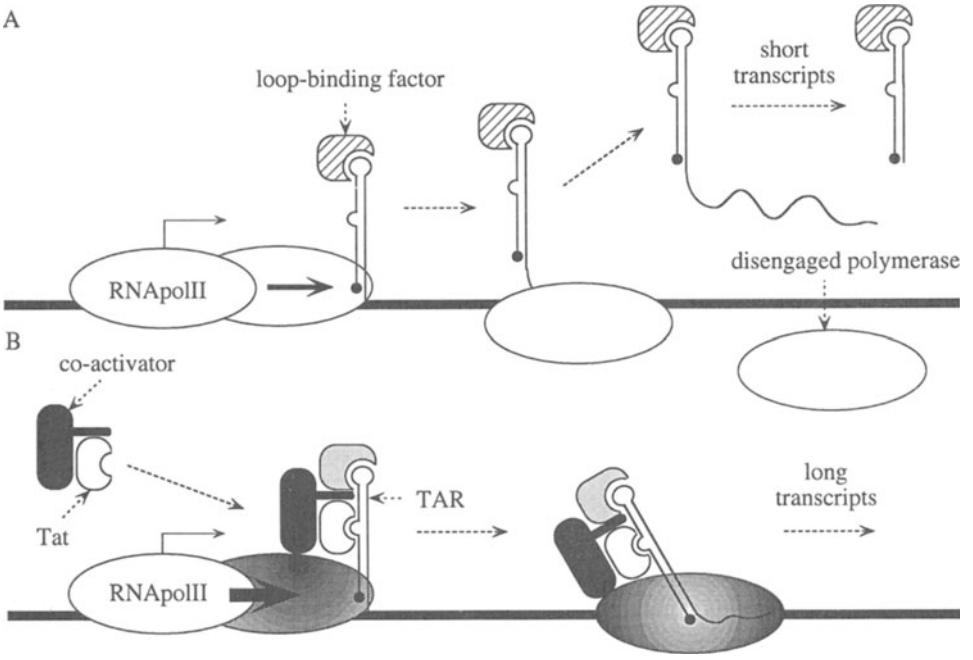


FIGURE 10. The mechanism of Tat *trans*-activation. (A) Cellular RNA polymerase II (RNApol II) and associated factors are assembled and start copying the viral genome. Cellular proteins also interact with TAR. However, in the absence of Tat, there is no modification of transcription complexes so that little to no processive transcription is observed. Most transcription complexes stall and disengage from the DNA template, thus releasing prematurely terminated RNA, which is degraded by cellular RNases to the stable TAR RNA stem-loop. These are called "short transcripts." (B) In the presence of Tat, its co-activators and cellular loop-binding proteins, Tat interacts with TAR and the RNA polymerase II to increase the processivity and competence of transcription complexes, which quickly copy the viral genome. This is a very specific and rapid process. Released are long polyadenylated transcripts that are differentially spliced and transported.

purified by affinity chromatography and this protein binds to the C-terminal half of Tat (Desai *et al.*, 1991). It restores high levels of Tat *trans*-activation in rodent cells. Although the rodent defect described by a number of investigators maps to the human chromosome 12, the location of the gene that codes for this 32-kDa protein is unknown. Other proteins that might be important for Tat *trans*-activation are the previously mentioned TRP-1/185, TRP-2, and the double-stranded RNA-dependent kinase (Jones and Peterlin,1994). Additionally, Tat can bind to Sp1 (Jeang *et al.*, 1993), a 42-kDa serine/threonine kinase (Herrmann and Rice, 1993), and TATA-binding protein (Kashanchi *et al.*, 1994). Some of these proteins are dispensable for Tat *trans*-activation and it remains to be seen which ones can function as Tat coactivators (see Chapter 2, this volume). Since a protein encoded by the human chromosome 12 is required for tethering Tat to TAR *in vivo* and since a cellular coactivator also interacts with the activation domain of Tat, it is possible that there are several proteins that mediate effects of Tat (Peterlin *et al.*, 1993; Jones and Peterlin, 1994).

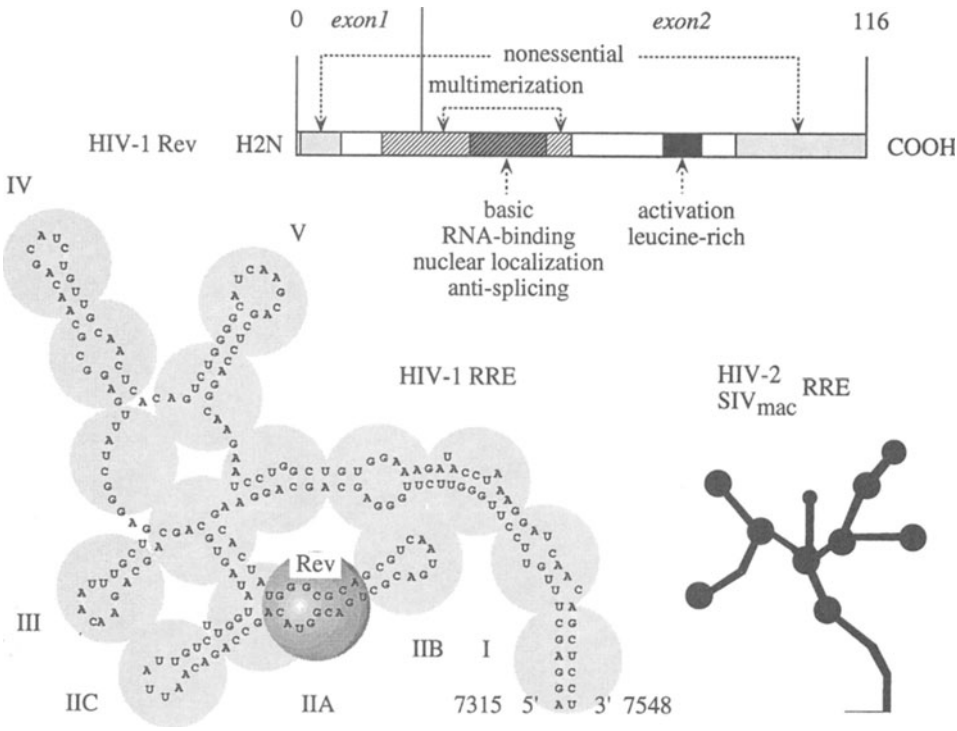


FIGURE 11. The structure of Rev. Most essential components of Rev are encoded by the second exon, which contains the multimerization, RNA-binding, and activation domains of Rev. Rev has a high affinity binding site in the stem-loop IIB (SLIIB), which is indicated by the heavy shading of Rev. However, after its binding to SLIIB, Rev multimerizes on the RRE so that greater than 20 and possibly more Revs coat the entire RNA structure. Although an RRE of 233 nucleotides is presented, recent evidence suggests that the secondary structure may extend both in the 5′ and 3′ directions (Gait and Karn, 1993). RRE of HIV-2 and SIV_{mac} is also schematized. The structure of the RRE also consists of many stem-loops and bulges. Experiments suggest that all retroviral Rev proteins function by similar mechanisms.

C. Rev

Rev contains 116 amino acids and measures 19 kDa (Fig. 11) (Cullen and Malim, 1991; Karn, 1991; Rosen and Dillon, 1991; Felber and Pavlakis, 1993; Gait and Karn, 1993; Parslow, 1993). It is translated from multiply spliced viral transcripts expressed early in the viral replicative cycle (Fig. 2). Although both exons flanking the *env* gene code for Rev, only the second exon contains important functional information (Fig. 11). After its translation, Rev can be phosphorylated on a serine near the C-terminus, but this phosphorylation has no known function (Rosen and Dillon, 1991). The nuclear localization sequence Asn^{40}-Arg^{41}-Arg^{42}-Arg^{43}-Arg^{44}-Trp^{45} directs Rev to the nucleus and to the nucleolus in cells (Cochrane *et al.*, 1990; Olsen *et al.*, 1991). Rev binds to the Rev response element (RRE) and multimerizes on the RNA. The RRE forms an RNA structure in the middle of the *env* gene (Malim *et al.*, 1989b).

Although no direct structural data are available, Rev has a number of functional domains (Fig. 11). Flanking its RNA-binding domain from amino acids 35 to 51, which forms an α-helix, are two oligomerization domains (Gait and Karn, 1993; Parslow, 1993; Tan *et al.*, 1993). Near its C-terminus is the leucine-rich activation domain from amino acids 73 to 84 (Leu^{75} to Leu^{84}). Mutations in this domain have a *trans*-dominant negative phenotype and interfere with the function of Rev (Malim *et al.*, 1989a). Yet the RNA-binding domain that is basic and contains several arginines and lysines also has antisplicing activity *in vitro* (Kjems *et al.*, 1991).

RRE is a 250-nt-long RNA structure located at the junction of the SU and TM sequences of *env* (Cullen and Malim, 1991; Karn, 1991; Rosen and Dillon, 1991; Felber and Pavlakis, 1993; Gait and Karn, 1993; Parslow, 1993). It is composed of four RNA stem-loops on a long stable stem with a predicted free energy of −110 kcal (Fig. 11) (Cullen and Malim, 1991). Rev binds with high affinity to SLII (Zapp and Green, 1989; Gait and Karn, 1993; Parslow, 1993). The activity of RRE is position-independent but orientation-dependent (Felber and Pavlakis, 1993; Gait and Karn, 1993; Parslow, 1993). RRE of HIV-2/SIV is slightly shorter (219 nt), is located 3′ to SU sequences, and has a different arrangement of stem-loops (Le *et al.*, 1990). It is of note that Rev of HIV-1 is active on RREs of all three viruses, but Rev of HIV-2/SIV only acts on its homologous RRE (Lewis *et al.*, 1990; Malim *et al.*, 1990; Sakai *et al.*, 1990).

Rev binds with high affinity to SLIIB (Fig. 11), whose RNA sequence and structure are important for this interaction. At low concentrations, Rev forms monomers, but higher-order complexes are observed with higher amounts of the protein (Fig. 12) (Olsen *et al.*, 1990; Malim and Cullen, 1991). These complexes form not only because of oligomerization domains of Rev, but also because multiple lower-affinity sites for Rev exist on the RRE and elsewhere on the RNA. Additionally, with high concentrations of Rev, long polymers, which appear as tubelike structures, have been observed in the presence and absence of RNA by electron microscopy (Heaphy *et al.*, 1991). In the presence of RNA, the nucleic acid is contained within these structures.

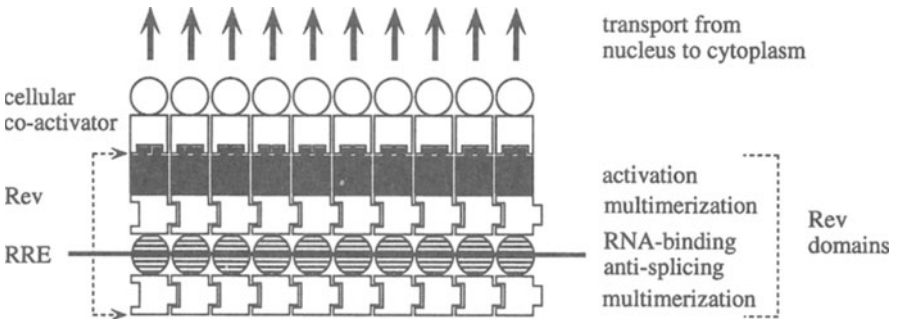


FIGURE 12. A schematic representation of Rev polymers. Different domains of Rev are diagrammed as interlocking boxes, which polymerize on the RNA. Multiple multimerization domains are required for the coating of RNA by Rev from its initial high affinity binding site (SLIIB). The activation domain of Rev contains several leucine residues and is thought to contact the cellular protein that transports viral genomic and singly spliced RNA from the nucleus to the cytoplasm. Data have been presented that Rev coats the entire viral RNA and that its basic domain interferes with RNA splicing *in vitro*.

Rev is an essential viral protein that facilitates the appearance of singly spliced and unspliced viral transcripts in the cytoplasm (Cullen and Malim, 1991; Karn, 1991; Rosen and Dillon, 1991; Felber and Pavlakis, 1993; Gait and Karn, 1993; Parslow, 1993). In the absence of Rev, only multiply spliced viral transcripts are translated, and therefore no viral structural proteins, enzymes, or genomic RNA can be packaged into virions (Fig. 13). Additionally, Rev increases the stability of RRE-containing RNA in the nucleus which is otherwise degraded rapidly (Malim and Cullen, 1993). Moreover, Rev inhibits splicing *in vitro* (Kjems *et al.*, 1991). However, even in the absence of apparent splice sites, Rev transports viral RNA from the nucleus to the cytoplasm and increases the dissociation between RRE-containing RNA and polysomes, thereby facilitating the translation of viral RNA (Felber and Pavlakis, 1993). Since the basic domain is required for RNA-binding and antisplicing activities and the activation domain is responsible for the effector function of Rev, it is possible that splicing and transport are linked and that antisplicing and transport activities reside on the same molecule and contact two different cellular coactivators.

To elucidate its mechanism of action, several cellular proteins that interact with Rev have been described. Biochemical and genetic approaches led to the isolation of a protein of 38 kDa (B23), 55 kDa, eIF-5A, and p32, all which associate with Rev. The 38-kDa protein has been obtained by affinity chromatography and identified as B23 (Fankhauser *et al.*, 1991). B23 translocates ribosomal components across nuclear membranes, and RRE dissociates the Rev–B23 complex. Therefore, B23 is thought to release Rev from RRE in the cytoplasm, thus allowing Rev to return to the nucleus where it encounters new RRE-containing viral transcripts. Although the 55-kDa protein binds to RRE in the absence of Rev, it is thought to facilitate the transport function of Rev (Vaishnav *et al.*, 1991). Both Rev and p55 bind to RRE simultaneously. The elongation initiation factor-5A (eIA-5A) plays an important role in the initiation of translation of eukaryotic mRNA in the cytoplasm (Ruhl *et al.*, 1993). However, this protein is most abundant in the nucleus and near nuclear pores where it associates with the activation domain of Rev in the presence of nuclear membranes. The eIF-5A might facilitate the transport of RRE-containing RNA from the nucleus to the cytoplasm. Finally, p32 associates with the basic domain of Rev (Luo *et al.*, 1994). Since p32 copurifies with the alternative splicing factor (ASF/SF2) that commits HIV pre-RNA to splicing, p32 is an attractive candidate for the protein that mediates the activity of Rev on splicing. Whether there are other proteins or whether any protein so far identified is the correct coactivator of Rev is unknown.

IV. VIRAL STRUCTURAL PROTEINS AND ENZYMES

As all viral transcripts accumulate in the cytoplasm, HIV structural proteins, enzymes, and accessory proteins are also synthesized (Greene, 1991; Haseltine, 1991; Bryant and Ratner, 1992; Clements and Wong-Staal, 1992; Luciw and Shacklett, 1993). In an ordered sequence of events, they lead to the budding of new virions from the plasma membrane (T cells) and possibly other membranes (e.g., on macrophages). The main players are Env, Gag-Pol, and Gag polyprotein precursor proteins.

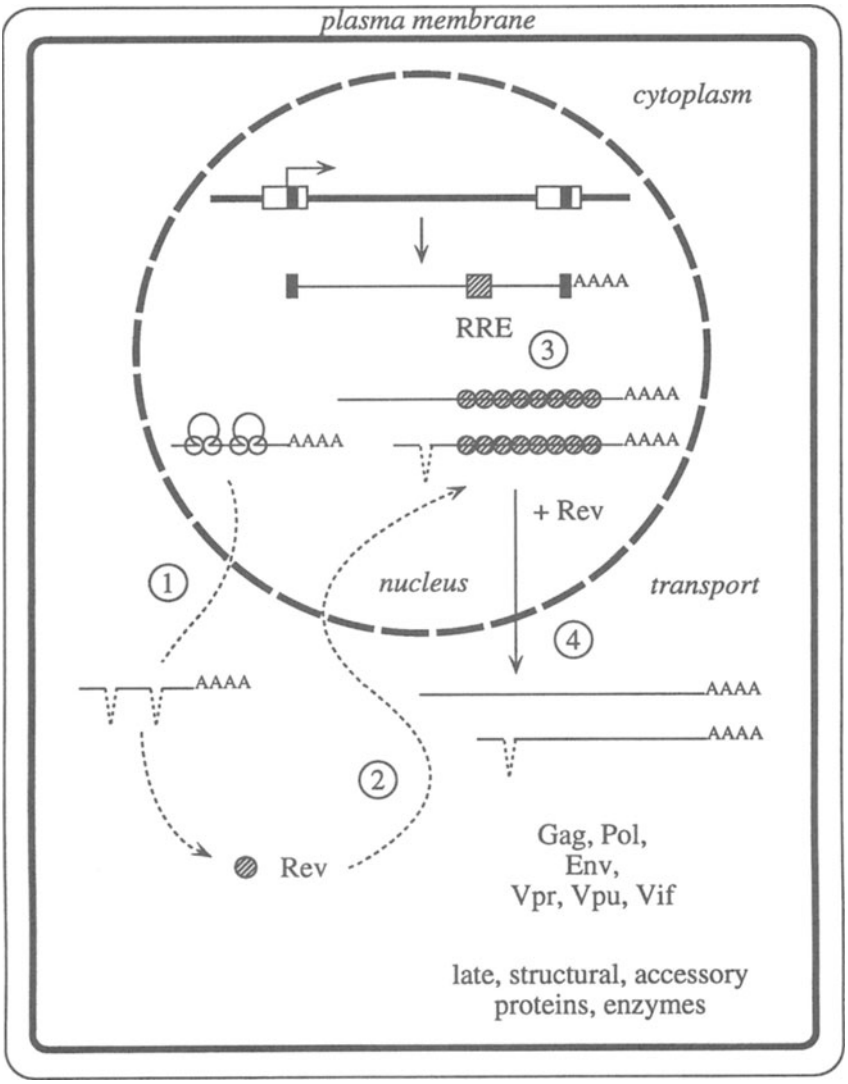


FIGURE 13. The mechanism of Rev action. By transporting unspliced and singly spliced transcripts from the nucleus to the cytoplasm, Rev shifts viral replication from the early to the late phase. (1) Rev is synthesized from multiply spliced viral transcripts, which are independent of Rev. (2) Rev then shuttles back to the nucleus and, at high concentrations, to the nucleolus. (3) By first binding to SLIIB and then coating the RRE, (4) Rev blocks further splicing events and transports RNA species, which contain the RRE, from the nucleus to the cytoplasm. Rev also increases the stability of long viral RNA species in the nucleus. In the cytoplasm, a cellular protein, which might be B23, removes Rev from the RNA.

A. Envelope

The HIV envelope is composed of two subunits: the 120-kDa surface (gp120, SU) and the 41-kDa transmembrane (gp41, TM) proteins (Doms *et al.*, 1993; Klasse *et al.*, 1993). It is translated from singly spliced viral transcripts whose expression depends on Rev (Fig. 2). The polyprotein Env precursor is synthesized first. This is a protein of 160 kDa (gp160) that is extensively glycosylated in its transit through the Golgi apparatus (Fig. 14). There it is also cleaved into gp120 and gp41 subunits by a cellular endopeptidase, a serine protease that may represent a mammalian streptolysin (Barr *et al.*, 1991). This proteolysis occurs after a stretch of three basic amino acids at the end of the gp120 SU domain (Hunter and Swanstrom, 1990).

However, prior to the proteolytic step, the Env precursor attaches to the endoplasmic reticulum (ER) via its signal peptide (Fig. 14) (Doms *et al.*, 1993). After the protein has entered the lumen of the ER, the signal peptide is removed. The stop transfer sequence is located in the middle of the transmembrane domain of gp160 (Fig. 15). After this step, oligosaccarides are added that consist first of mannose

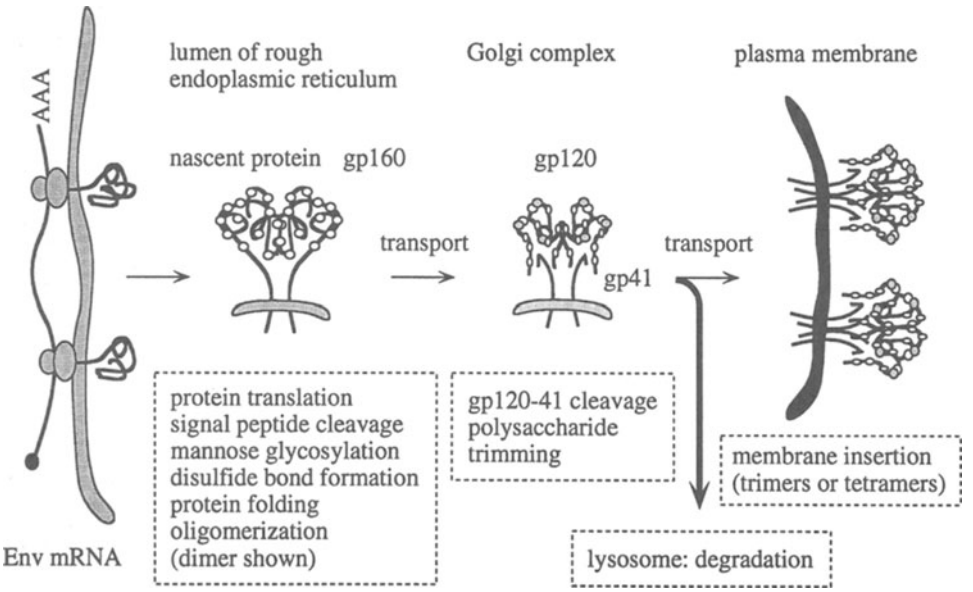


FIGURE 14. Processing of Env. Cellular mechanisms transport Env from the lumen of the rough endoplasmic reticulum to the plasma membrane. The gp160 precursor is translated from singly spliced viral transcripts and is inserted into the lumen of the rough ER where the signal peptide is cleaved. gp160 is glycosylated with mannose sugars and disulfide bonds are formed. With the help of cellular proteins, gp160 folds and forms dimers, trimers, and tetramers. Partially glycosylated proteins are transported to the Golgi apparatus, where more complex sugars are added and where gp160 is cleaved to gp120 and gp41 subunits. Most of Env is then degraded in lysosomes and only a very small fraction of the protein is inserted into the plasma membrane. About 72 different projections of multimeric forms of gp120 and 41 are found on the virion. In addition to being incorporated into the budding virion during morphogenesis, gp120 is also released from the cell-surface. (Modified from Luciw and Shacklett, 1993.)

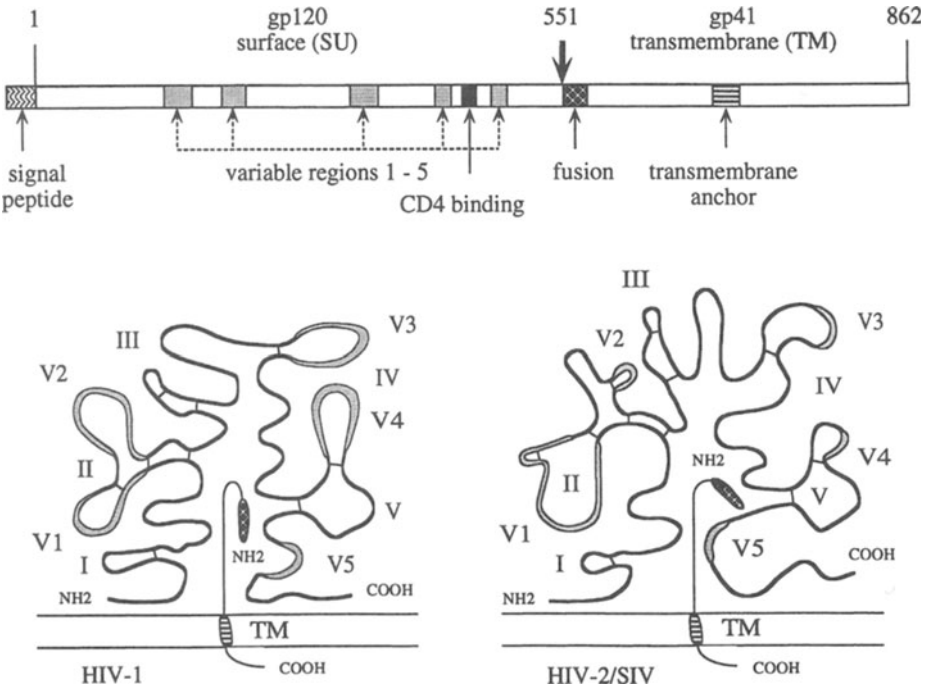


FIGURE 15. Viral envelope. The Env protein of HIV-1 is composed of two subunits, which are processed by a cellular protease into gp120 surface (SU) and gp41 transmembrane (TM) glycoproteins. Their gp160 precursor is translated from singly spliced viral transcripts that are Rev-dependent. A signal peptide is found at the N-terminus of gp120. Between variable regions V4 and V5 of gp120 is the CD4-binding domain. At the NH2-terminus of gp41 is a fusion domain that is required for viral entry into cells. A hydrophobic transmembrane anchor, which inserts gp41 into cellular and viral membranes, is located in the middle of the protein. The folding of HIV-1 and HIV-2/SIV Env glycoproteins is purely hypothetical and has been derived using monoclonal antibodies against various Env epitopes. In both Env proteins the fusion domain of gp41 is hidden in the belly of gp120, whose hypervariable domains stick out and are accessible for protein–protein interactions with other proteins and antibodies. Of special importance is the V3 loop, which is essential for fusion. However, the region that binds CD4 is region V between V4 and V5 domains. Disulfide bonds are represented by short lines and disulfide bonded domains are numbered with roman numerals I through V. No carbohydrates are shown although gp120 is at least 45% carbohydrate in mass. (Modified from Myers *et al.*, 1993.)

sugars and then of *N*-acetyl glucosamine, galactose, fructose, and other complex carbohydrates. gp160 is the most glycosylated viral protein, and this glycosylation is important for the proper folding of gp120 and its recognition of CD4 (Doms *et al.*, 1993; Klasse *et al.*, 1993).

At this point gp160 exists either as a trimer or tetramer and this multimeric structure remains intact throughout the assembly of the virion (Earl *et al.*, 1990; Gelderblom, 1991; Schawaller *et al.*, 1989; Weiss, 1993). Although the function of the full-length glycoprotein is unknown, if gp160 cannot be cleaved, the virion is not

infectious (McCune *et al.*, 1988; Stein and Engelman, 1990). Presumably this lack of infectivity is because gp41 contains the fusion domain, which must be accessible for the viral entry into cells. The processing of gp120 and gp41 is relatively inefficient and over 90% of intracellular gp160 is targeted to lysosomes and destroyed prior to its export to the plasma membrane (Earl *et al.*, 1991; Willey *et al.*, 1988). It is noteworthy that Env synthesized by the vaccina virus construct directs viral maturation to the basolateral membranes rather than to the cell surface, which might explain the cell-to-cell spread of the virus rather than the release of virions as one form of the infection (Owens *et al.*, 1991).

On the surface of cells and virions, gp120 and gp41 multimeric and oligomeric complexes exist via noncovalent interactions (Fig. 14) (Daar and Ho, 1990; Doms *et al.*, 1993). However, there are multiple intra-gp120 disulfide bonds that keep the molecule in its proper conformation (Fig. 15) (Leonard *et al.*, 1990). Even though the predicted molecular weight of the polyprotein is 88 kDa, the actual molecular weight of 160 kDa can be explained by its extensive glycosylation (Allan *et al.*, 1985; Robey *et al.*, 1985). There are 24 glycosylation sites in gp120 and four in gp41 so that the fully modified envelope is at least 45% carbohydrate in mass (Geyer *et al.*, 1988; Mizuochi *et al.*, 1990). Most of these glycosylation sites are conserved between different HIV isolates. This extensive glycosylation has so far precluded crystallization and detailed structural analyses of the protein. However, antibodies have been used to map exposed epitopes (Moore *et al.*, 1994). A model of nine intrachain disulfide bonds in gp120 has emerged that defines functional domains that are dependent on their confirmation for the recognition of CD4 (Fig. 15) (McDougal *et al.*, 1986; Capon and Ward, 1991; Leonard *et al.*, 1990). gp41 contains two hydrophobic stretches of which one is the N-terminal fusion peptide and the other anchors Env in membranes (Gallaher *et al.*, 1989). In some HIV-2 and SIV strains, gp41 is prematurely truncated to a protein of 32 kDa; however, the significance of this is unknown (Hirsch *et al.*, 1989; Kodama *et al.*, 1989). gp120 has at least five hypervariable domains and is 551 amino acids in length (Fig. 15).

Interactions between gp120 and CD4 have been extensively studied and have an affinity of 4×10^{-9} mole (Lasky *et al.*, 1987; Leonard *et al.*, 1990). However, different strains have different affinities and gp120 of SIV and HIV-2, for example, bind CD4 much less avidly than gp120 of HIV-1 (Moore, 1990). Domains 3, 4, and 5 interact with CD4 and antibodies against hypervariable domains 4 and 5 and block gp120–CD4 complex formation. CR1 and 2 are sites on CD4 where gp120 binds (Capon and Ward, 1991).

Upon interacting with CD4, gp41 becomes exposed and allows for the fusion between the virion and the cell (see Section VII, Fig. 19). Additionally, cells expressing high amounts of gp120 and CD4 fuse, resulting in giant-cell syncytia. V3 is the immunodominant epitope that helps to determine cell tropism and cytopathicity of HIV (Klasse *et al.*, 1993; Moore and Nara, 1991). It consists of 35 amino acids that are disulfide-linked at the bottom of an exposed bulge (Leonard *et al.*, 1990). Gly^{317}-Pro^{318}-Gly^{319} residues confer β followed by α helical domains (LaRosa *et al.*, 1990; Myers *et al.*, 1993). Mutations in the Gly^{317}-Pro^{318}-Gly^{319} motif result in a normal pattern of viral production but yield noninfectious virions (Grimalia *et al.*, 1992). These mutants also do not induce syncytia. Additionally, basic amino acids and hydrophobic amino acids in positions 311 and 325 confer syncytium producing (SI) and nonsyncytium inducing (NSI) phenotypes, respectively (DeJong *et al.*, 1992). By

simply changing these amino acids, an SI virus can be changed to an NSI virus and vice versa. Additionally, exchanges of the V3 loop convert a macrophage tropic virus (nonacidic amino acids at position 325) to a T-cell tropic virus (acidic amino acid or alanine residue at position 325) (Milich *et al.*, 1993; Schulz *et al.*, 1993). It has also been suggested that V3 is cleaved by cellular proteases in its Gly^{317}-Pro^{318}-Gly^{319}-Arg^{320}-Ala^{321}-Pro^{322} sequence, which is similar to the Gly-Pro-Cys-Arg-Ala-Pro sequence in gypstatin, a protease inhibitor (Hattori *et al.*, 1989; Clements *et al.*, 1991). This cleavage of V3 by an endosomal protease might be an important step for viral entry (Moore and Nara, 1991). However, the recently suggested second receptor for HIV, CD26, which is an exopeptase, cannot cleave V3 and is an unlikely candidate for this cellular enzyme (Callebaut *et al.*, 1993). It is of interest that the MuLV envelope is proteolytically cleaved by CD13, which is not only an amino peptidase but also the receptor for the human corona virus (Yeager *et al.*, 1992). Likewise, the viral determinant of infectivity of Newcastle disease virus is a cellular protease (Gorman *et al.*, 1990; Weiss, 1993).

B. Protease

Protease (PR) contains 99 amino acids and measures 10 kDa (Skalka, 1989; Debouck, 1992). It is released as a true enzyme or zymogen from the Gag-Pol precursor, and this release and activation are autocatalytic (Fig. 4) (Luciw and Shacklett, 1993). Presumably Gag-Pol dimers allow the protease to form homodimers that are required for its proteolytic activity and cleavage of the Gag-Pol precursor both in *cis* and in *trans* (Navia and McKeever, 1990). It is possible that for some reason the *trans* proteolysis is preferred (Burstein *et al.*, 1991). PR dimers attack other sites in Gag-Pol polyproteins and a total four to seven cleaves occur that are required for viral replication (Skalka, 1989; Debouck, 1992).

The detailed structure of PR has been determined to less than 3Å resolution (Fig. 16). Multiple mutants of PR have also been characterized by X-ray crystallography (Miller *et al.*, 1989; Navia *et al.*, 1989; Skalka 1989; Wlodawer *et al.*, 1989; Swanstrom *et al.*, 1990; Debouck, 1992). PR is related to other aspartyl proteases because the sequence Asp-Thr/Ser-Gly is conserved in the active site of viral and cellular enzymes (Toh *et al.*, 1985). It only works as a dimer (Oroszlan and Luftig, 1990). In its center, it contains a structure composed of several β strands and α helixes, which form a small active groove covered by a flap that moves aside during proteolysis (Fig. 16) (Skalka, 1989; Swanstrom *et al.*, 1990; Debouck, 1992).

PR has exquisite cleavage specificity that has been determined by synthetic peptides of 7 to 10 amino acids (Skalka, 1989; Swanstrom *et al.*, 1990; Debouck, 1992). Both primary sequence and the structure of the substrate affect its enzymatic activity, which spans four amino acids upstream and three amino acids downstream of the cleavage site (Pettit *et al.*, 1991). Upstream amino acids are always hydrophobic and unbranched at the β carbon. Although the consensus sequence in Gag is Ser-Thr-Xaa-Yaa-Phe/Tyr-Pro-Zaa, others can contain Leu-Ala, Met-Met, Phe-Leu, and Leu-Phe linkages. It is unusual for PR to cleave both Xaa-Pro and peptide bonds involving a primary amino acid. This fact has led to the synthesis of PR inhibitors, and the best inhibitors have steric complementarity to the active site (Debouck, 1992).

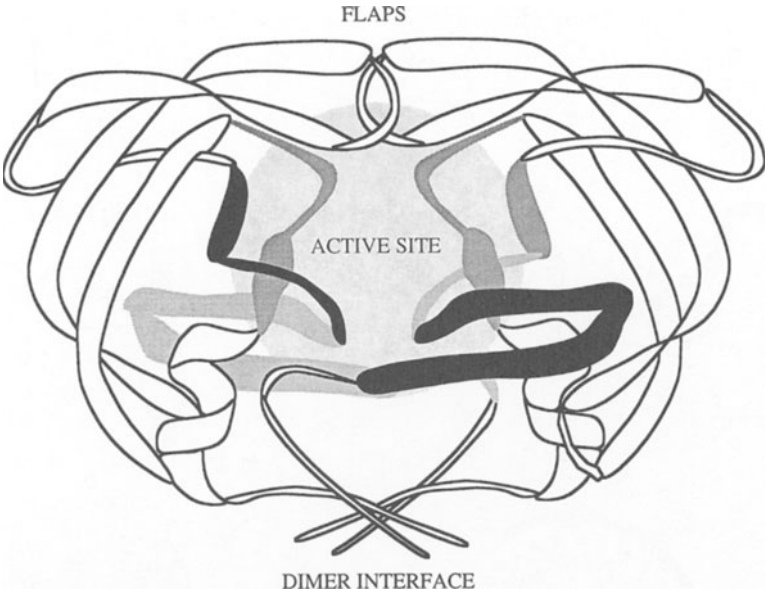
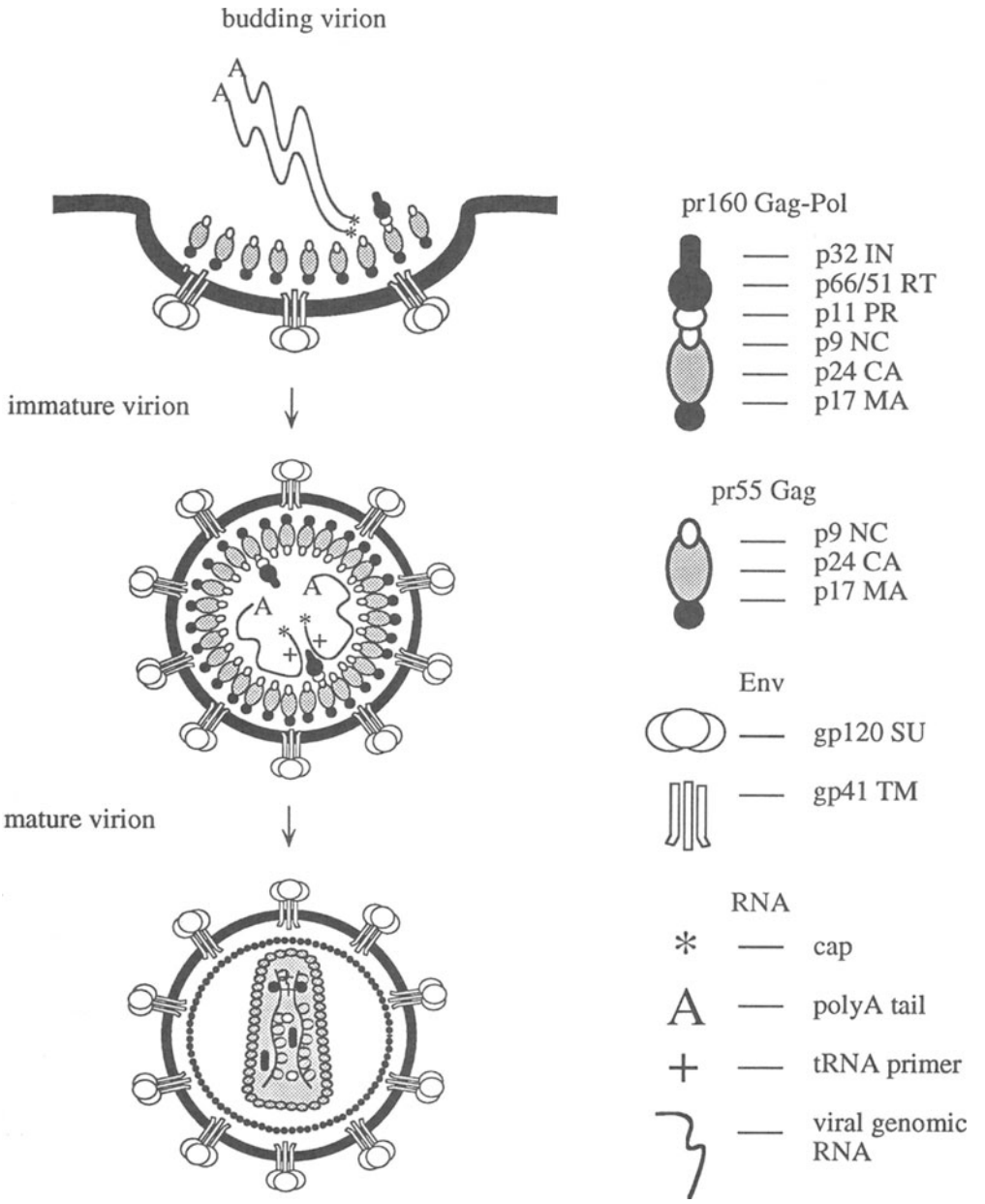


FIGURE 16. The structure of protease. PR is a 10-kDa protein and functions as a homodimer in cells. Its three-dimensional structure has been determined in exquisite detail by X-ray crystallography. PR looks like a crustacean. The active site is found at the base of the binding cleft, which is formed by interactions of two monomers. PR inhibitors bind in this site, which is shaded in this diagram. Dimers interface on the bottom where all four ends come together. On top of the active site are flaps that move upon binding of the appropriate substrate. The flaps and most of the contact points along the active site are formed by β strands. In this diagram, the folds that contact the active site are shaded either black or gray depending on their proximity to the page. (Modified from Debouck, 1992.)

C. Gag Proteins

Gag proteins are synthesized from a polyprotein pr55^{gag} precursor that consists of p17 (MA, matrix), p24 (CA, capsid), p7 (proline-rich), and p7 (NC, nucleocapsid) proteins that are translated from unspliced transcripts that depend on Rev (Fig. 4) (Wills and Craven, 1991; Henderson *et al.*, 1992; Morrow *et al.*, 1994). The pr55^{gag} precursor is synthesized at about 20-fold higher levels than the Gag-Pol polyprotein precursor (pr160$^{gag\text{-}pol}$) (Jacks, 1990). The subunits of Gag are proteolytically processed by the viral PR into subunits proteins that form the virion structure, i.e., both its outer and inner enclosures (Figs. 17 and 18) (Luciw and Shacklett, 1993).

The MA contains 131 amino acids and is composed of the 5′ sequences of the *gag* gene (Fig. 4). It is located in the mature virion between the core and Env glycoproteins (Gelderblom *et al.*, 1987a; Niedrig *et al.*, 1988). After the initiation of translation, the N-terminal methionine is removed and myristic acid is covalently added on the penultimate glycine (Schultz *et al.*, 1988). This reaction is catalyzed by the cellular *N*-myristyl transferase and is absolutely essential for viral infectivity (Gottlinger *et al.*, 1989; Bryant and Ratner, 1990). It targets intracellular cores to the cell membrane and is indistinguishable from that observed with Src and Nef proteins


budding virion
immature virion
mature virion
pr160 Gag-Pol
p32 IN
p66/51 RT
p11 PR
p9 NC
p24 CA
p17 MA
pr55 Gag
p9 NC
p24 CA
p17 MA
Env
gp120 SU
gp41 TM
RNA
cap
polyA tail
tRNA primer
viral genomic RNA

(Gheysen *et al.*, 1989; Andreassen *et al.*, 1990). MA is also posttranscriptionally phosphorylated on a serine residue without known function (Henderson *et al.*, 1990). Small deletions in the N-terminal domain of MA do not affect virion assembly and release but do diminish the inclusion of Env into the virion so that virions are not infectious (Yu *et al.*, 1992). Further C-terminal mutants of MA have a dominant negative phenotype and prevent viral propagation (Trono *et al.*, 1989). It is unknown which sequences in MA interact with gp41 (Dorfman *et al.*, 1994). Via its nuclear localization signal, MA also facilitates the translocation of uncoated viral particles form the cytoplasm to the nucleus of infected cells in the absence of cell division (Bukrinsky *et al.*, 1993).

The CA protein is a 244 amino acid hydrophobic protein that forms the core or capsid of the virion (Figs. 4, 17 and 18). Two cleavages by viral protease are required to release this 24 kDa protein, which is further phosphorylated on a serine residue (Henderson *et al.*, 1992). Again, the role of this phosphorylation is unclear. CA assembles *in vitro* after its expression in *Escherichia coli*, and one can observe either rodlike structures or irregular spheres (Prongay *et al.*, 1990). However, they do not resemble cone-shaped cores that are found in the virion. Thus, there must be other proteins that create the final shape of the capsid.

The NC protein is a 70 amino acid protein from the 3′ end of Gag (Fig. 4). It contains basic and hydrophilic domains and binds to genomic RNA in the virion core. However, this binding is not very specific (Karpel *et al.*, 1987). Within NC, there are two copies of the Zn^{2+} finger motif, of which the second is partially dispensable for function (Berg, 1990). The binding of Zn^{2+} is coordinated, and upon metal binding there is a change in the confirmation of NC as confirmed by circular dichroism spectroscopy (Surovoy *et al.*, 1993). The function of NC is to bind the packaging signal ψ and sequences within *gag* of full-length viral transcripts and to facilitate the dimerization of viral RNA in the virion (Darlix *et al.*, 1990; Weiss *et al.*, 1992). The Zn^{2+} finger binds double-stranded RNA, and this binding is essential for the packaging of RNA into virions (Aldovini and Young, 1990; Gorelick *et al.*, 1990). NC might also play a role in virion uncoating and might influence the copying of RNA to DNA during viral entry.

The morphogenesis of the virion at the cell surface occurs in an orchestrated

←

FIGURE 17. Virion assembly. A complicated series of events begins with the translation and expression of $pr160^{gag\text{-}pol}$. The myristylated Gag portion attaches to the plasma membrane and pulls the viral RNA behind via NC. By this time the oligomers have been transported to the plasma membrane via the *trans*-Golgi apparatus and there may be interactions between gp41 and MA. NC interacts with the ψ sequence located near the 5′ end of the viral genomic RNA. During this process, which occurs near the cell surface, dimers of $pr160^{gag\text{-}pol}$ come together and activate the protease, which then cleaves Gag and Pol precursors into subunit proteins. The processed Gag proteins interact with each other so that all the subunits are incorporated into the virion and that two copies of viral RNA are also attached. Cellular $tRNA^{lys}$ primer is also bound to the RNA. Other proteins that are pulled along are Vpr, Vif, and Vpu. Cellular proteins are also incorporated into the lipid bilayer. After the virion has partially formed, the maturation process continues. Thus, MA is localized between the core and Env, CA forms the cone shape core, and NC associates with the viral RNA. PR, RT, and IN are present together with Vpr, Vpu, and Vif. Different components of the polyprotein Env precursor and RNA structures are diagrammed to the right. (Modified from Luciw and Shacklett, 1993.)

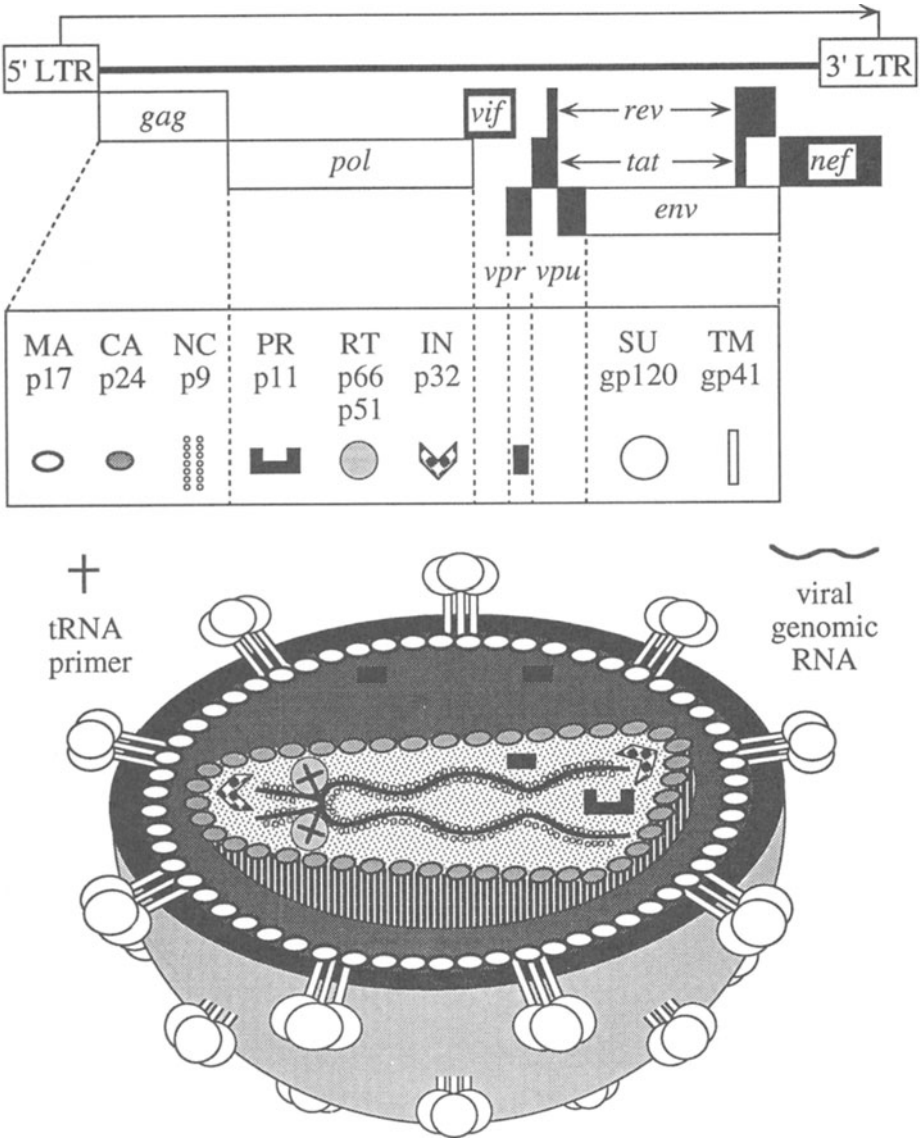


FIGURE 18. The virion. Below the HIV-1 genome are drawn viral proteins, which are incorporated into the virion. Abundant proteins are presented, but the less abundant Vif and Vpu are not included. Also, Tev is not drawn on the HIV-1 genome. The exact positions of viral enzymes like PR, RT, and IN in the viral core have not been determined, nor have the locations of Vif, Vpr, and Vpu. Moreover, Nef, Tat, and Ref are not detected in the virion. (Modified from Luciw and Shacklett, 1993.)

and sequential manner where PR is activated and starts cleaving the polyprotein Gag precursor, which is myristylated at the N-terminus and which holds the viral RNA at its C-terminus (Bryant and Ratner, 1990; Gelderblom, 1991; Doms *et al.*, 1993; Morrow *et al.*, 1994). By proteolysis *in situ*, these components then selectively polymerize, forming the outer and inner walls of the virion particle, which holds the viral RNA in its center via NC (Fig. 18) (Gelderblom, 1991).

V. VIRION ASSEMBLY

The first step in virion assembly involves interaction between Gag and Gag-Pol precursor proteins (Gelderblom, 1991; Luciw and Shacklett, 1993). Electron-dense crescents at the plasma membrane include $pr55^{gag}$ and $pr160^{gag\text{-}pol}$ and genomic viral RNA (Delchambre *et al.*, 1989; Gheysen *et al.*, 1989). The ψ, or the encapsidation (E) element, binds the NC domain of Gag (Lever *et al.*, 1991; Linial and Miller, 1990). The ψ contains 120 nt between the 5′ LTR and the start of the *gag* gene (Aldovini and Young, 1990; Lever *et al.*, 1991). This sequence forms a stable RNA structure and also intermolecular RNA homoduplexes with the other viral RNA strand (Harrison and Lever, 1992). As oligomers of Env are inserted into the plasma membrane, the viral RNA–protein complex is extruded through the cell surface (Fig. 17). This process adds membrane proteins, such as β2 microglobulin and class I and II major histocompatibility complex (MHC) molecules into the lipid bilayer of the virion (Gelderblom *et al.*, 1987b; Arthur et al, 1992). In this process, the MA domain of Gag interacts with gp41 (TM) of Env (Dorfman *et al.*, 1994). As the virion matures, protease forms dimers in *trans* and cleaves Gag and Gag-Pol polyproteins into subunit proteins (Fig. 17). This results in the mature virion with MA, CA, NC, and p7 of Gag; PR, PT, and IN of Pol; two strands of viral RNA; Vpr, which binds to Gag; and possibly small amounts of Vif (Fig. 18) (Gelderblom, 1991).

The targeting of the virion to the plasma membrane is due to the N-terminal myristylation of Gag (Gottlinger *et al.*, 1989; Bryant and Ratner, 1990; Smith *et al.*, 1993). Additionally, interactions between CA of Gag and cyclophilins might facilitate this movement (Luban *et al.*, 1993). Incorporation of the $tRNA^{lys}$ primer in virions involves RT (Mak *et al.*, 1994). That all these Env, Gag, Pol, and Vpr proteins remain associated during the budding process implies that they have independent protein–protein interactions. Thus, it is not surprising that deletion mutants of Gag have strong *trans*-dominant phenotypes that inhibit viral replication (Trono *et al.*, 1989).

VI. VIRION STRUCTURE

The structure of the HIV virion has been determined by electron microscopy and studied by biochemical and immunochemical methods (Fig. 18) (Gelderblom *et al.*, 1989; Grief *et al.*, 1989; Gelderblom, 1991). The virion is about 110 nm in diameter. On the outside, it has a lipid bilayer to which the Env glycoproteins are attached. This lipid bilayer is wrapped around the matrix, and inside the matrix there is a core that is about 100 nm long and 40 to 60 and 20 nm wide at the free and narrow ends, respectively (Marx *et al.*, 1988; Gelderblom *et al.*, 1989). The narrow

end is attached by a proteinacious structure to the matrix (Marx *et al.*, 1988; Gelderblom *et al.*, 1989; Hoglund *et al.*, 1992). On the envelope there are 9- to 10-nm knobs or spikes with triangular symmetry (Marx *et al.*, 1988; Gelderblom *et al.*, 1989; Hoglund *et al.*, 1992). There are a total of 72 of these spikes on each virion (Gelderblom, 1991). These knobs have a narrow stalk by which they are attached to the lipid bilayer. Other cell surface proteins of macrophages and T cells have also been observed on the virion surface, such as β2 microglobulin, α and β chains of class II MHC, class I MHC molecules, and so forth (Arthur *et al.*, 1992; Gelderblom *et al.*, 1987b).

In the virion core there are two single strands of viral genomic RNA to which the $tRNA^{lys}$ primer is hydrogen-bonded at the 5′ end. Furthermore, the RNAs are held together at their 5′ ends through hydrogen bonds and short antiparallel sequences (Darlix *et al.*, 1990; Coffin, 1991; Marquet *et al.*, 1991, 1994). There is also some viral DNA produced by partial reverse transcription (Trono, 1992). Furthermore, there are cleavage products of Gag and Gag-Pol polyproteins. For example, there are NC and p9 near the RNA, as well as RT, RNase H, Vpr, and possibly smaller amounts of Vpu (Fig. 18). CA forms the core shell and MA, which is myristylated, fills the gap between the virion envelope and the core. Both of these form the structure of the virion by their polymerization during viral budding (Fig. 17).

Enzymes encoded by the *pol* gene are less abundant than those from the Gag proteins and their precise topology in the virion has not yet been established. However, PR, RT, and IN are all present in the virion. They form a large multiprotein complex which together with viral genomic RNA is then released into infected cells following virion uncoating. Since MA and Vpr are also required for targeting the viral RNA and DNA into the nucleus and contain nuclear localization signals, they must also be associated with this large particle (Bukrinsky *et al.*, 1993).

VII. VIRAL ENTRY AND INTEGRATION

HIV enters cells by first binding to its receptor, CD4 (Fig. 19) (Dalgliesh *et al.*, 1984; Klatzmann *et al.*, 1984; Capon and Ward, 1991; Klasse *et al.*, 1993). This interaction, and possible proteases on the cell surface, expose gp41, which results in the fusion between the virus and the cell (Weiss, 1993; chapter 1, this volume). HIV enters via a pH-independent channel or organelle (Stein *et al.*, 1987; Sinangil *et al.*, 1988; McClure *et al.*, 1990). Since CD4 is sufficient for infection of all kinds of human but not rodent cells, the existence of a second, human receptor for HIV has been postulated (Weiss, 1933). Entry also requires the reductive cleavage of disulfide bonds of Env, since the inhibition of disulfide isomerase, which is located on the plasma membrane, blocks HIV infection (Ryser *et al.*, 1994).

Upon viral entry, viral uncoating proceeds simultaneously with the beginning of reverse transcription and translocation of the remaining viral particle from the cytoplasm to the nucleus (Varmus and Brown, 1989; Whitcomb and Hughes, 1992). Reverse transcription begins with the $tRNA^{lys}$ primer at the primer binding site on the positive strand (Fig. 20) (Luciw and Shacklett, 1993). Second-strand synthesis then proceeds from short template fragments generated by RNase H at the polypurine tracts (DeStefano *et al.*, 1991). Two strand transfers are required for this process (Peliska and Benkovic, 1992). This process also duplicates the R region at

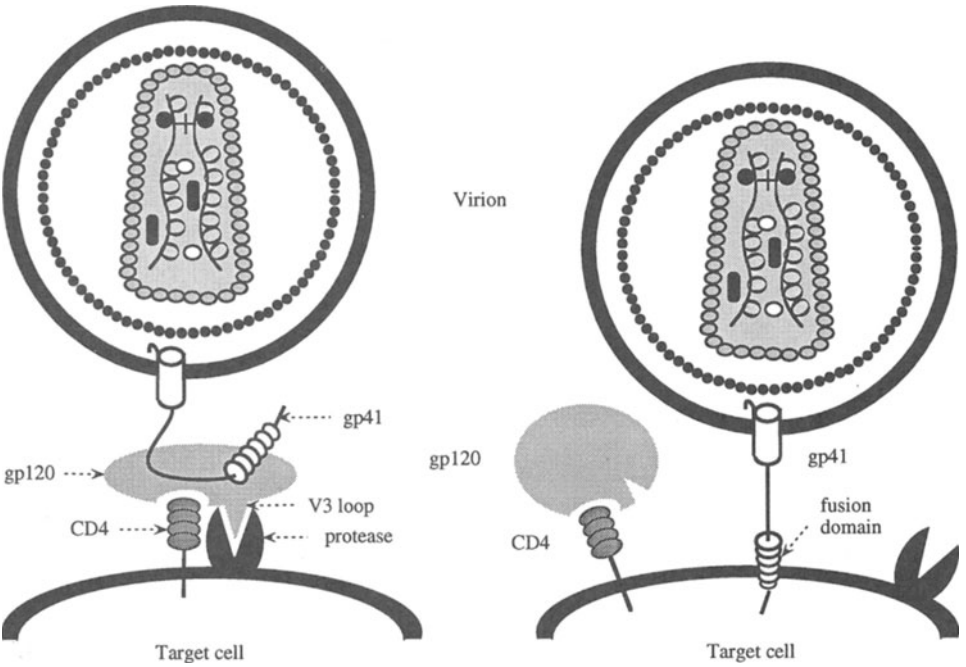


FIGURE 19. Viral entry. The virion interacts with the cell by binding of gp120 to CD4. This event changes the conformation of gp120, exposing the fusion domain of gp41. The process might be helped by a cellular protease, which cleaves the V3 loop.The membranes of the virus and the cell fuse, releasing the virion core into cells. A complicated series of uncoating steps follows. Unknown signals also activate RT to start copying the viral RNA to DNA. Vif facilitates these steps. MA and Vpr contribute to the cytoplasmic to nuclear translocation of these viral protein–nucleic acid complexes, which later integrate into the host cellular DNA. Note that prior to binding of gp120 to CD4, gp41 is buried in gp120 and holds it on the virion. (Modified from Luciw and Shacklett, 1993.)

both ends of the viral LTRs. Partially replicated intermediates and double-stranded DNA are translocated to the nucleus with the help of MA and Vpr proteins (Bukrinsky *et al.*, 1993). Full-length linear double-stranded DNA integrates into the host cell genome with the help of IN (Varmus and Brown, 1989; Brown, 1990; Goff, 1992; Whitcomb and Hughes, 1992).

A. Reverse Transcriptase and RNase H

Reverse transcriptase contains 440 amino acids and measures 51 kDa (Fig. 21) (Jacobo-Molina and Arnold, 1991; Skalka and Goff, 1993). RT–RNase H polyprotein contains 560 amino acids and measure 66 kDa. Both are translated from unspliced viral transcripts that are Rev-dependent and appear late in the viral replicative cycle (Figs. 2 and 4). The translation of RT requires translational frameshifting so that RT is only 5% as abundant as Gag (Jacks, 1990). It is processed by the viral protease during virion assembly and is packaged into virions.

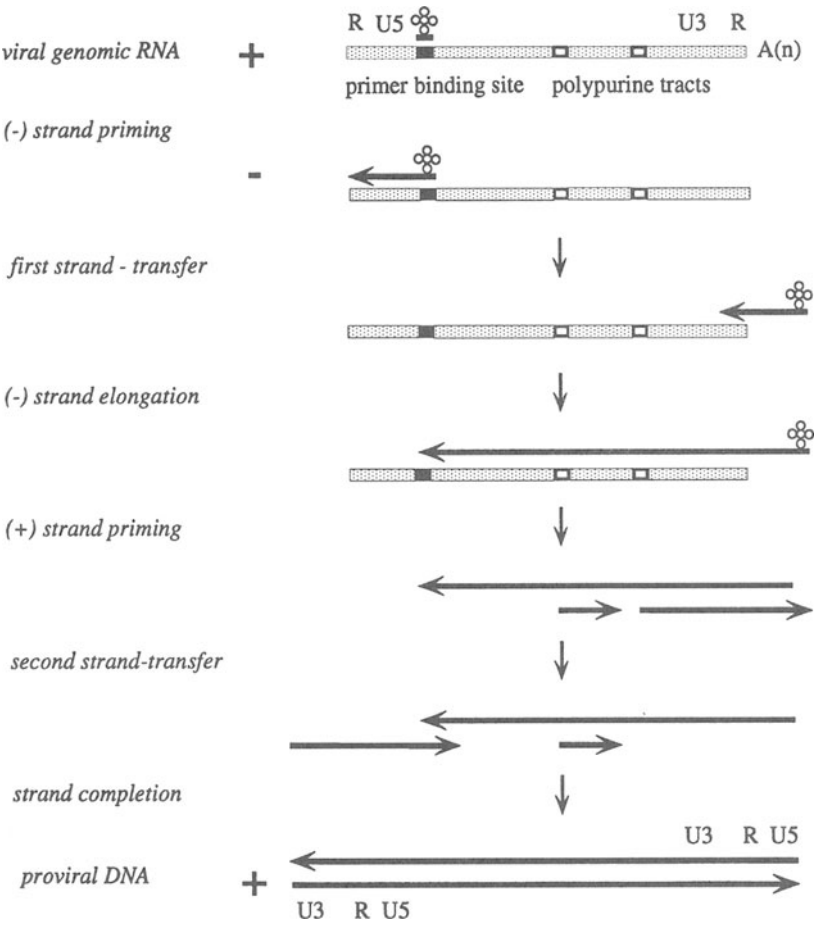


FIGURE 20. Mechanism of reverse transcription. The viral RNA contains the plus-strand information and is diagrammed as a gray line. The $tRNA^{lys}$ primer binds to the primer binding site and primes reverse transcription which produces the single-stranded DNA of the negative strand. The synthesis of the second strand is primed by the short DNA template revealed by RNase H activity on the polypurine tract that borders U3 sequences of transcribed sequences. Negative-strand elongation then proceeds on the viral RNA in the 5′ direction. Thus, by the combination of RNase H activity and reverse transcription of RNA and DNA, both strands are made efficiently. PolyA of the viral genomic RNA is represented as A(n), the primer binding site is represented by a black rectangle, and the polypurine tracts are represented by open rectangles. It should be noted that because the R sequences serve as the first strand transfer sequences, they duplicate the LTRs so that U3RU5 and U5RU3 sequences are found on both ends of the proviral DNA.

The biosynthesis of RT and RT–RNase H is a two-step process. First, the $pr160^{gag\text{-}pol}$ is cleaved by PR to p66 which forms a homodimer (LeGrice, 1993). Next, because the structure of the p66 subunits is not the same in this homodimer, one subunit is cleaved by PR to produce p51 (Arnold *et al.*, 1992; Kohlstaedt *et al.*, 1992). The connecting peptide in the other p66 is shielded from further proteolysis. Mixed

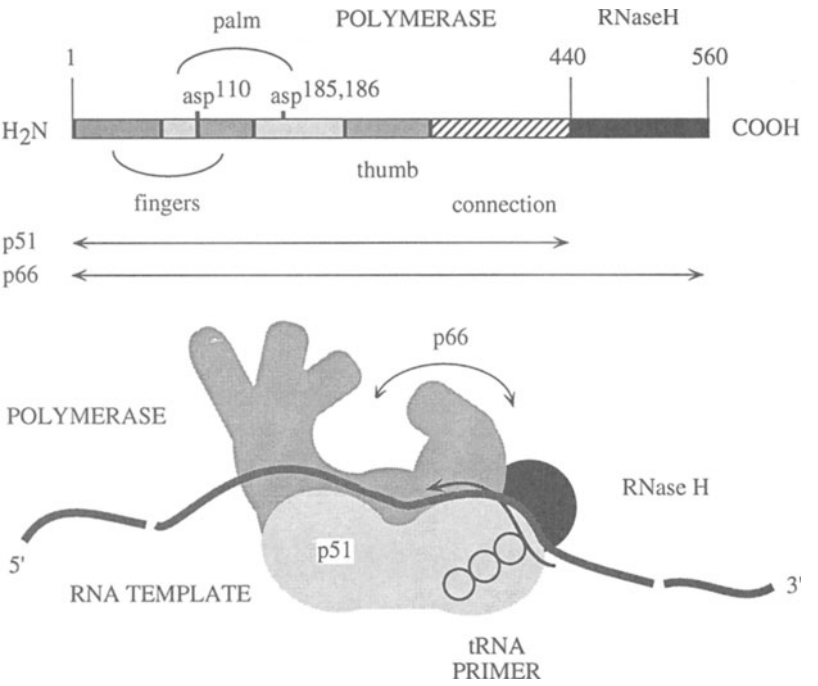


FIGURE 21. Viral polymerase. The HIV polymerase contains both RT and RNase H activities. As a polyprotein it is called p66 and RT is called p51. RT is composed of several domains that resemble a right hand by X-ray crystallography. Residues active in catalysis are labeled (asp^{110}, asp^{185}-asp^{186}). Connection links RT to RNase H. In the palm of the hand are diagrammed the RNA (dark line) and the $tRNA^{lys}$ primer, which binds to the p51 subunit (light gray structure). The thumb and fingers form the active site for the polymerase (dark gray structure), whereas the RNase H removes RNA from RNA–DNA hybrids (black circle). Structurally, p66 and p51 are quite distinct and it is the removal of the RNase H that is the differentiating feature of this structure. (Modified from Kohlstaedt *et al.*, 1992.)

heterodimers result that have the same N-termini, but only the p66 subunit also contains RNase H.

The structure of RT and RNase H has been determined by X-ray crystallography; the entire complex with monoclonal antibodies (MAb) and duplex DNA template primer to 7 Å resolution (Arnold *et al.*, 1992), RT with nonnucleoside inhibitor nevirapine to 3.5 Å resolution (Kohlstaedt *et al.*, 1992), and the small RNase H subunit to 2.5 Å resolution (Davies *et al.*, 1991). What emerges is that the mixer heterodimer resembles the human right hand with fingers, palm, and thumb (Fig. 21). The p66 forms the top of the hand which contains four subdomains side-by-side that are elongated and measure $110 \times 30 \times 45$ Å. RNase H forms a separate domain at the thumb (Kohlstaedt *et al.*, 1992; Jacobo-Molina *et al.*, 1993). The central two domains appear very similar to the Klenow fragment of *E. coli* DNA polymerase I, whose catalytic site is also found in a cleft. Tyr^{183}-Met^{184}-Asp^{186} are conserved between all retroviral RTs and DNA polymerases (Doolittle *et al.*, 1989; Delarue

et al., 1990; Xiong and Eickbush, 1990). It is thought that the connecting domain holds the template primer in place and then the transcription occurs near the Tyr^{183}-Met^{184}-Asp^{185}-Asp^{186} sequence.

The structure of p51, which contains only RT, is different from p66, although they are derived from the same precursor (Arnold *et al.*, 1992; Kohlstaedt *et al.*, 1992). Mainly, p51 has no cleft and aspartic residues are not available for catalysis. Moreover, p66 and p51 interact in a head-to-tail arrangement and therefore form an asymmetric dimer. A different surface of the same subdomain faces the long groove in both proteins. The RNase H is folded into a five-stranded mixed β sheet which is flanked by four α helixes (Davies *et al.*, 1991). Its structure is very similar to the bacterial RNase H (Yang *et al.*, 1990). Two divalent metal ions are bound by four acidic residues Asp^{443}, Glu^{478}, Asp^{498}, and Asp^{549}, which are conserved among all RNase H proteins and are essential for their function (Doolittle *et al.*, 1989; Yang *et al.*, 1990).

RT is required to copy viral RNA to single-stranded DNA and then to covert the single-stranded DNA to double-stranded DNA. Unlike other RTs, RT or HIV and SIV does not have any dUTPase activity (Elder *et al.*, 1992). The DNA synthesis that occurs in the cleft results in RNA–DNA heterodimers, which is where the RNA template is cleaved at about 16 to 18 nt downstream from the site of synthesis (Arnold *et al.*, 1992). This length can be explained by the distance between active sites of RT and RNase H, which measures about 20 amino acids in the cleft. Thus, Asp^{185} and Asp^{186} of RT, which represent the active site, are separated from Asp^{443}, Glu^{478}, Asp^{498}, and Asp^{549} of RNase H. Catalytic sites initiate polymerization of both RNA and DNA templates on RT.

Since RT lacks a proofreading mechanism, it generates viral sequence polymorphisms (Katz and Skalka, 1990; Coffin, 1992). The measured *in vitro* misincorporation rate of RT is 1 in 1700 to 1 in 4000 nt which is 10- to 100-fold higher than the misincorporation rate of bacterial and mammalian DNA polymerases (Battula and Loeb, 1974; Preston *et al.*, 1988; Roberts *et al.*, 1988, 1989). This translates to five to ten misincorporations per HIV genome per replication *in vivo* (Pathak and Temin, 1990). Both deletions and point mutations are produced, and these are maintained by selective pressures, such as immune responses and cellular tropism, which result in variant viruses also known as quasi-species in the host (Wain-Hobson, 1992).

The nature of reverse transcription and the tertiary structure of the transcription complexes have led to various interventions in the process of viral transcription (Broder *et al.*, 1990; Mitsuya *et al.*, 1990; De Clercq, 1992; Erice and Balfour, 1994; Neuzil, 1994). Most useful drugs are dideoxynucleotide triphosphate (ddNTPs) analogues like 3′-azido-3′-deoxythymidine (AZT), dideoxycytine (ddC), and dideoxyinosine (ddI), which cause premature chain termination by binding preferentially to RT. However, rapid mutations in RT arise that render these drugs ineffective. For example, RT resistant to AZT and ddI contains mutations in Met^{41} and Asp^{67}, Asp^{69}, Lys^{70}, Leu^{74}, Thr^{215}, to Lys^{219} (Larder and Kemp, 1989; St. Clair *et al.*, 1991). Other drugs that have been used and are nonnucleoside analogues include nevirapine, which binds to both primer template and ddNTPs. Phosphonoacetic acid, fuchsin, rifabutin, tetrahydroimidazobenzodiazepinones (TIBO), actinomycin D, and polymeric compounds such as dextran sulfate and phosphorothioates are other anti-RT agents (Broder *et al.*, 1990; Mitsuya *et al.*, 1990; De Clercq, 1992; Erice and Balfour, 1994; Neuzil, 1994). However, since resistance to all of these drugs can be observed

even when given simultaneously, there is great need for other rational drug design and new inhibitors of RT–RNase H.

B. Integrase

Integrase (IN) is a 31- to 32-kDa protein that is processed by viral protease from the C-terminus of the $pr160^{gag\text{-}pol}$ during virion assembly (Figs. 1, 3, 4, and 22) (Brown, 1990; Goff, 1992; Whitcomb and Hughes, 1992). It is translated from unspliced viral RNA by translation frameshifting and is also packaged into virions. Viral IN is active in bacteria, yeast, insect cells, and *in vitro*. Its N-terminal region is highly conserved among retroviruses and contains a Zn^{2+}-finger motif, which might

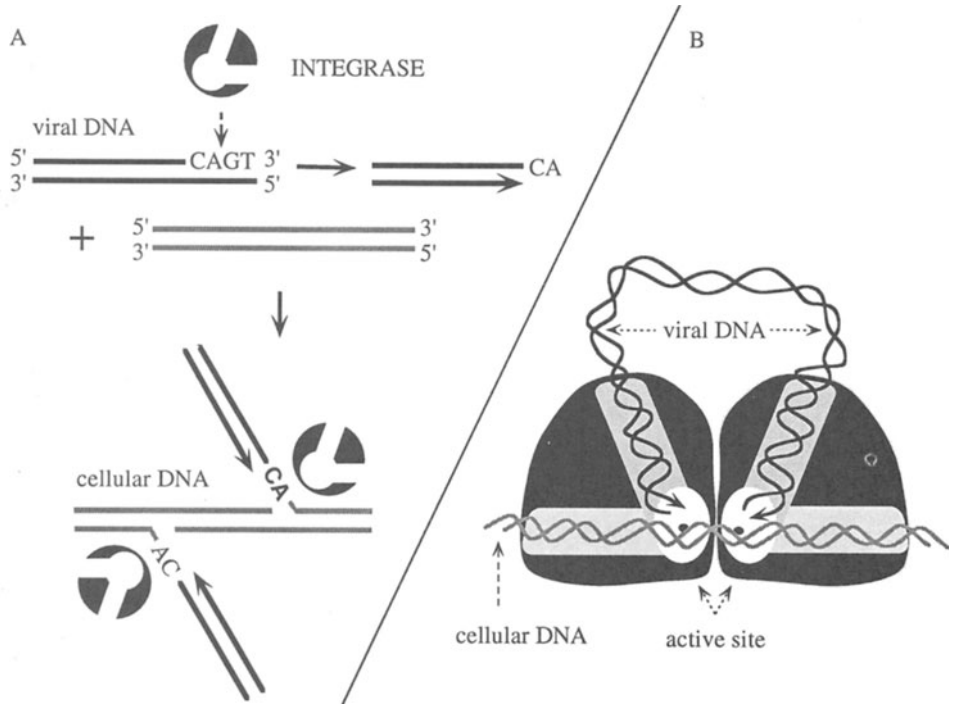


FIGURE 22. Viral integrase. (A) *In vitro* reaction for IN activity uses double-stranded DNA as a substrate for the 3′-end processing and the target for strand transfer. Here, black lines denote oligonucleotides that are processed by IN and the target DNA is represented by gray lines. IN recognizes a highly conserved nucleotide CA and a dinucleotide in the substrate and removes nucleotides immediately 3′ to the CA sequence. This 3′-processed oligonucleotide is later inserted into the target double-stranded DNA. Purified enzyme without any other proteins can mediate this reaction in forward and reverse directions. (B) In performing this process, IN dimers hold both processed and target DNA near the active site, presumably via binding sites for both strands. The active site interacts at the junction of the two DNA-binding domains such that free ends of DNA are opposed to each other. In this figure, cellular DNA refers to target DNA and viral DNA refers to the DNA inserted into the host genome. (Modified from Vincent *et al.*, 1993.)

be required for DNA binding (Burke *et al.*, 1992). It complexes Zn^{2+} and forms homodimers in solution (Sherman and Fyfe, 1990; Jones *et al.*, 1992; Vincent *et al.*, 1993). The model of IN is of a single active site with separate binding sites for viral and cellular DNA (Fig. 22B). Each subunit attaches to a single viral DNA end (Fig. 22). It is the dimeric form of IN that mediates integration (Vincent *et al.*, 1993). For further analysis of IN and DNA strands, we will need structural resolution by X-ray or NMR spectroscopies, which has been impossible due to the low solubility of IN when expressed in *E. coli*.

Forward and reverse reactions mediated by IN have been studied *in vitro* (Cragie *et al.*, 1990; Katz and Skalka, 1990; Engelman *et al.*, 1991). Short duplex oligonucleotides that make the ends of viral DNA are incorporated into larger DNA targets (Luciw and Leung, 1992). Integrase cleaves thymidine (TT) dinucleotides from the 3′ end of the duplex attachment (att) sites, which can be as short as 12 to 40 nt. After cleavage, they produce staggered cuts of 5 nt in duplex DNA, and then a strand transfer reaction is catalyzed where recessed 3′ hydroxyl ends are joined to 5′ phosphorylated ends in the target DNA (Buchman and Craigie, 1990). Cleavage and joining steps are separable and the energy for the reaction preexists in the bond not required for joining the new oligonucleotides to the target DNA (Engelman *et al.*, 1991; Vink *et al.*, 1991a). Similar one-step *trans*-esterification reactions have been described for phage λ DNA when it attaches itself to target DNA (Mizuuchi and Adzuma, 1991). The conserved CA dinucleotide next to the 3′ site is important for the cleavage and strand transfer reactions. Moreover, the IN recognizes 6 to 9 nt in the terminal LTR of viral DNA so that integration is specific for viral DNA and not for cellular DNA (LaFemina *et al.*, 1991; Vink *et al.*, 1991b; Sherman *et al.*, 1992). Also, att sites form other retroviruses are not cleaved. The reverse reaction in this integration is also catalyzed *in vitro*, and release of substrates into DNA has been observed (Chow *et al.*, 1992). The forward and reverse directions of its activity imply that IN is a true enzyme and has a concerted mechanism of action.

VIII. ACCESSORY PROTEINS

HIV codes for three small accessory proteins: Vif, Vpr, and Vpu (Terwilliger, 1992; Gibbs and Desrosiers, 1993; Morrow *et al.*, 1994). In HIV-2 and some SIV strains, *vpr* is duplicated and is called *vpx* (Fig. 1) (Myers *et al.*, 1993). HIV-2 and most SIV strains also do not contain *vpu*. Since their expression is regulated by Rev from singly spliced viral transcripts, they represent late proteins (Fig. 2). None of them are found in other nonprimate lentiviruses (Luciw and Leung, 1992). They are involved in the morphogenesis and infectivity of virions (Fig. 23), and their function has been appreciated best in peripheral blood mononuclear cells and in animals rather than in cultured cell lines (Terwilliger, 1992; Gibbs and Desrosiers, 1993; Morrow *et al.*, 1994). Furthermore, their effects are more pronounced on the cell-free spread of HIV than on its cell-to-cell transmission. Vpr (Vpx) and possibly small amounts of Vif are also packaged in the virion (Fig. 18).

A. Vif

Vif contains 193 amino acids and measures between 23 and 27 kDa (Kan *et al.*, 1986; Lee *et al.*, 1986; Sodroski *et al.*, 1986). It is translated from 5 kb singly spliced

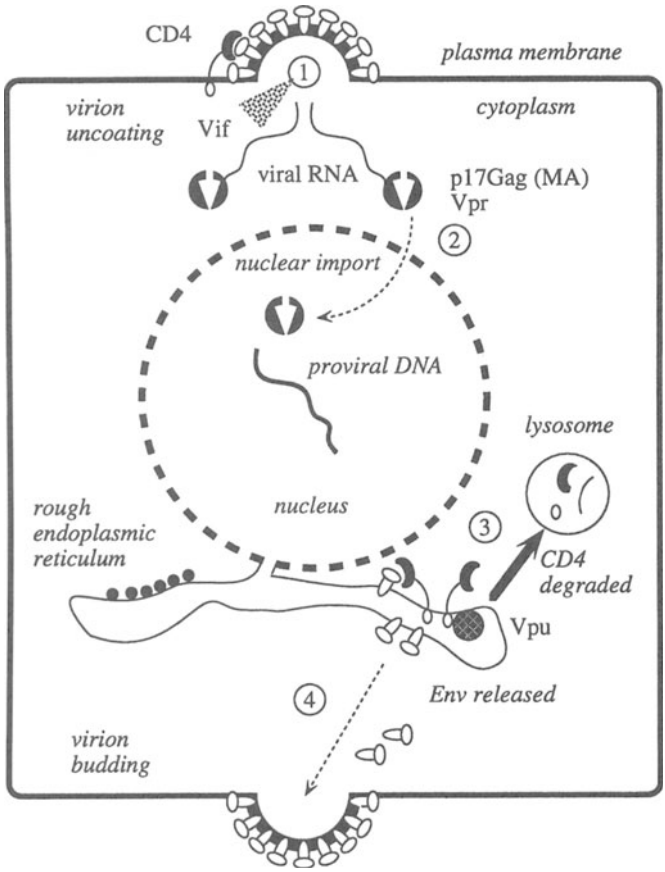


FIGURE 23. HIV accessory proteins. Vif, Vpr, and to a lesser extent Vpu are contained in the virion and are required for high levels of viral replication in $CD4^+$ lymphocytes. They are less essential for viral replication and cell-to-cell spread of the virus in replicating tissue culture cells. Their functions have become better clarified. (1) Vif is required for the efficient uncoating and release of viral RNA and the synthesis of viral DNA in the cytoplasm. (2) Vpr helps p17 Gag transport viral particles containing Gag proteins, RT, and viral RNA and DNA from the cytoplasm to the nucleus, which occurs even in the absence of cell division to integrate proviral DNA into the host genome. (3) Vpu releases CD4 from the synthesized gp160 and leads to rapid degradation of CD4 in lysosomes. This and other effects of Vpu enable more complete and faster maturation of viral particles from the translation of proteins in the ER to the morphogenesis of virions on the cell surface.

transcripts from coding sequences 3′ to the *pol* gene (Fig. 2). Vif is detected in the cytoplasm of infected cells and possibly in small amounts in virions, whose structure is intact in the absence of Vif (Fisher *et al.*, 1987). It contains approximately equal numbers of hydrophobic and hydrophilic amino acids and is not glycosylated (Oberste and Gonda, 1992).

HIV infectivity is reduced 1000-fold in some $CD4^+$ T cells in the absence of Vif. However, cell-to-cell transmission of the virus remains normal (Sodroski *et al.*, 1986; Fisher *et al.*, 1987; Strebel *et al.*, 1987). Since some cells show no effect of Vif, they must contain a Vif-complementing factor (Fan and Peden, 1992; Gabuzda *et al.*,

1992). Three different phenotypes of Vif have been described. First, Vif is required for the complete synthesis of viral DNA, which could be due to its effects on the internalization of the nucleocapsid, virion uncoating, activation of RT, or RNA processing (Fig. 23) (Sova and Volsky, 1993; von Schwedler *et al.*, 1993). The second proposes that Vif is a cysteinyl protease, which could play a role in the processing or conformation of Env (Guy *et al.*, 1991). Finally, Vif might be required in the maturation process, since in its absence only low amounts of Env are expressed on the virion (Sakai *et al.*, 1993).

B. Vpr

Vpr contains 100 amino acids and measures 15 kDa (Franchini *et al.*, 1988; Henderson *et al.*, 1988; Yu *et al.*, 1988). Like Vif, Vpr is translated from singly spliced transcripts from coding sequences between *vif* and *tat* (Fig. 2) (Garrett *et al.*, 1991; Schwartz *et al.*, 1991). Whereas HIV-1 and SIV_{cpz} contains only *vpr*, HIV-2, SIV_{mac}, and SIV_{smm} contain both *vpr* and *vpx*, and SIV_{agm} contains only *vpx* (Fukasawa *et al.*, 1988; Myers *et al.*, 1993). However, *vpr* and *vpx* share common sequences and might be duplications of each other (Tristem *et al.*, 1990, 1992). Vpr is colocalized at the inner cell membrane with p27 (Kappes *et al.*, 1993) or in the nucleus with p7 (Y.L. Lu *et al.*, 1993; Paxton *et al.*, 1993). Large amounts of Vpr are also packaged into virions (Wong-Staal *et al.*, 1987; Franchini *et al.*, 1988; Henderson *et al.*, 1988; Cohen *et al.*, 1990; Yu *et al.*, 1988, 1990). Not only is its incorporation independent of core assembly (Sato *et al.*, 1990), but the morphology of the virion is unaffected by Vpr.

Vpr is dispensible for viral replication in most cell lines but not in primary cells (Guyader *et al.*, 1987; Hu *et al.*, 1989; Shibata *et al.*, 1990; Kappes *et al.*, 1991; Marcon *et al.*, 1991) and is critical for viremia and progression to AIDS in monkey (Lang *et al.*, 1993). These effects are most pronounced at low multiplicity of infection and in macrophages (Ogawa *et al.*, 1989; Hattori *et al.*, 1990). In part, this could be due to effects of Vpr and MA on the translocation of uncoated viral complexes from the cytoplasm to the nucleus in the absence of cell division (Fig. 23) (Bukrinsky *et al.*, 1992). Its arginine-rich C-terminal domain might represent the nuclear localization of Vpr (X. Lu *et al.*, 1993; Zhao *et al.*, 1994). Additionally, Vpr can activate transcription nonspecifically. For example, expression from the SV40 early promoter and LTRs of MuLV, avian sarcoma leukosis virus (ASLV), HIV, and HIV-1 are increased three- to tenfold in the presence of Vpr (Cohen *et al.*, 1990). Vpr can also block the proliferation of human rhabdomyosarcoma cells and induce its differentiation to muscle cells (Levy *et al.*, 1993). Thus, Vpr might change the cellular environment so that it is more permissive for viral replication.

C. Vpu

Vpu contains 80 amino acids and measures 16 kDa (Cohen *et al.*, 1988; Strebel *et al.*, 1988). Found only in HIV-1 and SIV_{cpz}, it is translated from coding sequences between *tat/rev* and *env* genes on 5-kb singly spliced transcripts (Fig. 2) (Cohen *et al.*, 1988; Matsuda *et al.*, 1988; Strebel *et al.*, 1988; Cochrane *et al.*, 1990). Fifty base pairs of Vpu overlap *env* but in a different translation frame, and both Vpu and Env

are translated from the same mRNA (Schwartz *et al.*, 1990). Many viral strains have defects in their *vpu* genes (Myers *et al.*, 1993). Vpu contains hydrophobic ends and a hydrophilic center, and self-aggregates, is phosphorylated on one or more serines, and is an integral membrane protein (Strebel *et al.*, 1988, 1989). However, it is not found in virions and does not affect their morphology (Strebel *et al.*, 1989).

Whereas more viruses are released from cells in the presence of Vpu, numerous viral particles are found in intracytoplasmic vesicles in the absence of Vpu (Klimkait *et al.*, 1990). This process is due in part to the trapping of gp160 by CD4 in the ER (Willey *et al.*, 1994). Consequently, levels of CD4 on the cell surface and the processing of gp160 to gp120 and gp41 are reduced. Vpu decreases the number of these gp160–CD4 complexes and the stability of CD4 from 6 h to 12 min (Willey *et al.*, 1994). The 32 intracytoplasmic residues of CD4, specifically Leu^{414}-Ser^{415}-Lys^{416}-Lys^{417}-Thr^{418}, and the hydrophilic C-terminal domain of Vpu are required for these effects (Chen *et al.*, 1993; Willey *et al.*, 1994). It is of interest that Leu^{414} is also required for the decreased expression of CD4 on the cell surface by Nef (Aiken *et al.*, 1994). Thus, Vpu facilitates the export of Env and the budding of mature virions (Fig. 23).

IX. MOLECULAR PATHOGENESIS

From the description of viral genes and proteins, it is clear that most are essential for viral replication, cytopathology, and progression to disease (Fauci, 1991; Luciw and Shacklett, 1993; Morrow *et al.*, 1994). All regulatory proteins, Gag, Pol, and Env are critical for the viral replicative cycle. Even accessory proteins are important for high levels of viremia in primary cells and monkeys (Luciw *et al.*, 1992; Lang *et al.*, 1993). Interfering with the function of any of these proteins should greatly retard disease progression and might cure AIDS. Combination therapies, i.e., targeting more than one viral protein or process, might hold even more promise, especially with the high rates of mutations introduced by RT (Broder *et al.*, 1990; Mitsuya *et al.*, 1990; Erice and Balfour, 1994; Neuzil, 1994).

HIV can kill an infected cell either metabolically or by forming giant-cell syncytia with other $CD4^+$ cells (Levy, 1993). The main contributors to these processes are Env, CD4, and high levels of viral replication. However, other viral proteins are also able to adversely affect cellular processes. Nef leads to the apoptosis of infected immature T cells (Bonyhadi *et al.*, 1993; Baur *et al.*, 1994). Tat supports the growth of Kaposi's sarcoma cells in culture (Ensoli *et al.*, 1990). Transgenic mice expressing Tat also exhibit skin lesions (Vogel *et al.*, 1988). Additionally, Tat can activate the transcription of several cellular and other viral genes (Peterlin *et al.*, 1993; Jones and Peterlin, 1994). Since some of them encode lymphokines and cytokines, this function of Tat might adversely affect immune responses and the proliferation of mononuclear cells. It has been reported that Rev can alter the splicing patterns of host transcripts, for example, of the T-cell antigen receptor (Koga *et al.*, 1988). Vpr can cause cellular differentiation, as was demonstrated with rhabdomyosarcoma cells (Levy *et al.*, 1993).

Most viral proteins can be degraded to antigenic peptides, which elicit host immune responses (Phillips *et al.*, 1991). In part, this process is beneficial to the host, because the immune system tries to rid itself of this pathogen. However, in

some instances, when viral sequences resemble host proteins, for example, Nef and Env and class II MHC, this might lead to autoimmunity and increased symptomatology (Milich *et al.*, 1993; Shugars *et al.*, 1993; see Chapter 7, this volume). Paradoxically, yet other viral peptides block antiviral immune responses (Klenerman *et al.*, 1994).

Other aspects of HIV pathogenesis relate to the viral replicative cycle itself. For progression to disease, HIV has to accomplish the following: enter the host, replicate in cells of the immune system, elicit acute symptoms, establish latency and a reservoir of the virus, replicate again and disseminate new virions, and, finally, lead to end-organ damage (Levy, 1993). An intricate interplay between the virus and the host persists throughout the infection (Pantaleo *et al.*, 1993). Latency, at least in part, might require that cells are not activated and that Nef and Tat are not expressed (Adams *et al.*, 1994). However, the dissemination of the virus relies most heavily on Env. Although an NSI, macrophage tropic virus might be easier to pass from individual to individual; an SI, T-lymphocyte tropic virus is thought to lead to the depletion of $CD4^+$ cells and immunodeficiency (Schuitemaker *et al.*, 1992; Chapter 1, this volume). Macrophage trophic viruses might also be more likely to cause central nervous system disturbances (Gendelman and Morahan, 1992).

Although cellular cytopathology can be examined with HIV-1, studies on the progression to disease required animal models and SIV (Gardner and Luciw, 1992; Gardner *et al.*, 1994). Here, different hypotheses concerning viral proteins, cellular cofactors, and immune responses can be studied directly. Moreover, attenuated vaccines, which represent the most promising solution to the AIDS crisis, can best be developed in this model (Desrosiers, 1992). In the meantime, the Achilles heel of the virus might be revealed by detailed structural and mechanistic studies of all viral proteins.

ACKNOWLEDGMENTS. I thank Michael Armanini for expert secretarial assistance, Jay Levy and Paul Luciw for critical comments on the manuscript and help with the figures, and members of my laboratory for their indulgence. This chapter is dedicated to my wife Anne and my children Anton Alexander and Sebastian Bogomir.

X. REFERENCES

Adams, M., Sharmeen, L., Kimpton, J., Romeo, J. M., Garcia, J. V., Peterlin, B. M., Groudine, M., and Emerman, M., 1994, Cellular latency in human immunodeficiency virus-infected individuals with high CD4 levels can be detected by the presence of promoter-proximal transcripts. *Proc. Natl. Acad. Sci. USA* **91**:3862.

Aiken, C., Konner, J., Landau, N. R., Lenburg, M. E., and Trono, D., 1994, Nef induces CD4 endocytosis: Requirement for a critical dileucine motif in the membrane-proximal CD4 cytoplasmic domain, *Cell* **76**:853.

Aldovini, A., and Young, R. A., 1990, Mutations of RNA and protein sequences involved in HIV-1 packaging in production of noninfectious virus, *J. Virol.* **64**:1920.

Allan, J. S., Coligan, J. E., Barin, F., McLane, M. F., Sodroski, J. G., Rosen, C. A., Haseltine, W. A., Lee, T. H., and Essex, M., 1985, Major glycoprotein antigens that induce antibodies in AIDS patients are encoded by HTLV-III, *Science* **228**:1091.

Andreassen, H., Bohr, H., Bohr, J., Brunak, S., Bugge, T., Cotterill, R. M. J., Jacobsen, C., Kusk, P.,

Lautrup, B., Petersen, S. B., Saermark, T., and Ulrich, K., 1990, Analysis of the secondary structure of the human immunodeficiency virus (HIV) proteins p17, gp120, and gp41 by computer modeling based on neural network methods, *J. Acquir. Immune Defic. Syndr.* **3:**615.

Arnold, E., Jacobo-Molina, A., Nanni, R. G., Williams, R. L., Lu, X., Ding, J., Clark, A. D., Zhang, A., Ferris, A. L., Clark, P., Hizi, A., and Hughes, S. H., 1992, Structure of HIV-1 reverse transcriptase/DNA complex at 7 Å resolution showing active site locations, *Nature* **357:**85.

Arthur, L. O., Bess, J. W., Sowder, R. C., Benveniste, R. E., Mann, D. L., Chermann, J., and Henderson, L. E., 1992, Cellular proteins bound to immunodeficiency viruses: Implications for pathogenesis and vaccines, *Science* **258:**1935.

Barr, P. J., Mason, O. B., Landsberg, K. E., Wong, P. A., Kiefer, M. C., and Brake, A. J., 1991, cDNA and gene structure for a human subtilisin-like protease with cleavage specificity for paired basic amino acid residues, *DNA Cell Biol.* **10:**319.

Battula, N., and Loeb, L. A., 1974, The infidelity of avian myeloblastosis virus deoxyribonucleic acid polymerase in polynucleotide replication, *J. Biol. Chem.* **249:**4086.

Baur, A. S., Sawai, E. T., Dazin, P., Fantl, W. J., Cheng-Mayer, C., and Peterlin, B. M., 1994, HIV-1 Nef leads to inhibition or activation of T cells depending on its intracellular localization, *Immunity* **1:**373.

Berg, J. M., 1990, Zinc fingers and other metal-binding domains, *J. Biol. Chem.* **265:**6513.

Bonyhadi, M. L., Rabin, L., Salimi, S., Brown, D. A., Kosek, J., McCune, J. M., and Kaneshima, H., 1993, HIV induces thymus depletion *in vivo*, *Nature* **363:**728.

Braddock, M., Thorburn, A. M., Chambers, A., Elliott, G. D., Anderson, G. J., Kingsman, A. J., and Kingsman, S. M., 1990, A nuclear translational block imposed by the HIV-1 U3 region is relieved by the Tat-TAR interaction, *Cell* **62:**1123.

Broder, S., Mitsuya, H., Yarchoan, R., and Pavlakis, G. N., 1990, NIH conference. Antiretroviral therapy in AIDS, *Ann. Intern. Med.* **113:**604.

Brown, P. O., 1990, Integration of retroviral DNA, *Curr. Top. Microbiol. Immunol.* **157:**19.

Bryant, M., and Ratner, L., 1990, Myristoylation-dependent replication and assembly of human immunodeficiency virus I, *Proc. Natl. Acad. Sci. USA* **87:**523.

Bryant, M. L., and Ratner, L., 1992, Biology and molecular biology of human immunodeficiency virus, *Pediatr. Infect. Dis. J.* **11:**390.

Buchman, F. D., and Craigie, R., 1990, Activities of human immunodeficiency virus (HIV) integration protein *in vitro*: Specific cleavage and integration of HIV DNA, *Proc. Natl. Acad. Sci. USA* **88:**1339.

Bukrinsky, M. I., Sharova, N., Dempsey, M. P., Stanwick, T. L., Bukrinskaya, A. G., Haggerty, S., and Stevenson, M., 1992, Active nuclear import of human immunodeficiency virus type 1 preintegration complexes, *Proc. Natl. Acad. Sci. USA* **89:**6580.

Bukrinsky, M. I., Haggerty, S., Dempsey, M. P., Sharova, N., Adzhubel, A., Spitz, L., Lewis, P., Goldfarb, D., Emerman, M., and Stevenson, M., 1993, A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells, *Nature* **365:**666.

Burke, C. J., Sanyal, G., Bruner, M. W., Ryan, J. A., LaFemina, R. L., Robbins, H. L., Zeft, A. S., Middaugh, C. R., and Cordingley, M. G., 1992, Structural implications of spectroscopic characterization of a putative zinc-finger peptide from HIV-1 integrase, *J. Biol. Chem.* **267:**9639.

Burstein, H., Bizub, D., and Skalka, A. M., 1991, Assembly and processing of avian retroviral Gag polyproteins containing linked protease dimers, *J. Virol.* **65:**6165.

Callebaut, C., Krust, B., Jacotot, E., and Hovanessian, A. G., 1993, T cell activation antigen, CD26, as a cofactor for entry of HIV in $CD4^+$ cells, *Science* **262:**2045.

Capon, D. J., and Ward, R. H. R., 1991, The CD4–gp120 interaction and AIDS pathogenesis, *Annu. Rev. Immunol.* **9:**649.

Carroll, R., Martarano, L., and Derse, D., 1991, Identification of lentivirus Tat functional domains through generation of equine infectious anemia virus/human immunodeficiency virus type 1 *Tat* gene chimeras, *J. Virol.* **65:**3460.

Chamorro, M., Parkin, N., and Varmus, H. E., 1992, An RNA pseudoknot and an optimal heptameric shift site are required for highly efficient ribosomal frameshifting on a retroviral messenger RNA, *Proc. Natl. Acad. Sci. USA* **87:**713.

Chen, M. Y., Maldarelli, F., Karczewski, M. K., Willey, R. L., and Strebel, K., 1993, Human immunodeficiency virus type 1 Vpu protein induces degradation of CD4 *in vitro*: The cytoplasmic domain of CD4 contributes to Vpu sensitivity, *J. Virol.* **67**:3877.

Cheng-Mayer, C., Lannello, P., Shaw, K., Luciw, P. A., and Levy, J. A., 1989, Differential effects of Nef on HIV replication: Implications for viral pathogenesis in the host, *Science* **246**:1629.

Cherrington, J., and Ganem, D., 1992, Regulation of polyadenylation in human immunodeficiency virus (HIV): Contributions of promoter proximity and upstream sequences, *EMBO J.* **11**:1513.

Chow, S. A., Vincent, K. A., Ellison, V., and Brown, P. O., 1992, Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus, *Science* **255**:723.

Chowers, M. Y., Spina, C. A., Kwoh, T. J., Fitch, N. J., Richman, D. D., and Guatelli, J. C., 1994, Optimal infectivity *in vitro* of human immunodeficiency virus type 1 requires an intact *nef* gene, *J. Virol.* **68**:2906.

Clements, G. J., Price-Jones, M. J., Stephens, P. E., Sutton, C., Schulz, T. F., Clapham, P. R., McKeating, J. A., McClure, M. O., Thomason, S., Marsh, M., Kay, J., Weiss, R. A., and Moore, J. P., 1991, The V3 loops of the HIV-1 and HIV-2 surface glycoproteins contain proteolytic cleavage sites: A possible function in viral fusion, *AIDS Res. Hum. Retroviruses* **7**:3.

Clements, J. E., and Wong-Staal, F., 1992, Molecular biology of lentiviruses, *Semin. Virol.* **3**:137.

Cochrane, A. W., Perkins, A., and Rosen, C. A., 1990, Identification of sequences important in the nucleolar localization of human immunodeficiency virus Rev: Relevance of nucleolar localization to function, *J. Virol.* **64**:881.

Coffin, J. M., 1991, Retroviridae and their replication, in: *Fundamental Virology* (B. N. Fields and D. M. Knipe, eds.), p. 645, Raven Press, New York.

Coffin, J. M., 1992, Genetic diversity and evolution of retroviruses, *Curr. Topics Microbiol. Immunol.* **176**:143.

Cohen, E. A., Terwilliger, E. F., Sodroski, J. G., and Haseltine, W. A., 1988, Identification of a protein encoded by the *vpu* gene of HIV-1, *Nature* **334**:532.

Cohen, E. A., Terwilliger, E. F., Jalinoos, Y., Provix, J., Sodroski, J. G., and Haseltine, W. A., 1990, Identification of HIV-1 *vpr* product and function, *J. AIDS* **3**:11.

Cragie, R., Fujiwara, T., and F. Bushman, F., 1990, The IN protein of Moloney murine leukemia virus processes the viral DNA ends and accomplishes their integration *in vitro*, *Cell* **62**:829.

Cullen, B. R., 1991, Human immunodeficiency virus as a prototypic complex retrovirus, *J. Virol.* **65**:1053.

Cullen, B. R., 1992, Mechanism of action of regulatory proteins encoded by complex retroviruses, *Microbiol. Rev.* **56**:375.

Cullen, B. R., and Greene, W. C., 1989, Regulatory pathways governing HIV-1 replication, *Cell* **58**:423.

Cullen, B. R., and Malim, M. H., 1991, The HIV-1 Rev protein: Prototype of a novel class of eukaryotic post-transcriptional regulators, *Trends Biochem. Sci.* **16**:346.

Daar, E. S., and Ho, D. D., 1990, The structure and function of retroviral envelope glycoproteins, *Semin. Virol.* **1**:205.

Dalgliesh, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A., 1984, The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus, *Nature* **312**:763.

Darlix, J. L., Gabus, C., Nugeyre, M. T., Clavel, F., and Barré-Sinoussi, F., 1990, *Cis* elements and *trans* factors involved in the RNA dimerization of the human immunodeficiency virus HIV-1, *J. Mol. Biol.* **216**:689.

Davies, J. F., Hostomska, Z., Hostomsky, Z., Jordan, S. R., and Matthews, D. A., 1991, Crystal structure of the ribonuclease H domain of HIV-1 reverse transcriptase, *Science* **252**:88.

Debouck, C., 1992, The HIV-1 protease as a therapeutic target for AIDS, *AIDS Res. Hum. Retroviruses* **8**:153.

De Clercq, E., 1992, HIV inhibitors targeted at the reverse transcriptase, *AIDS Res. Hum. Retroviruses* **8**:119.

DeJong, J. J., Goudsmit, J., Keulen, W., Klaver, B., Krone, W., Tersmette, M., and DeRonde, A, 1992, Human immunodeficiency virus type 1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity, *J. Virol.* **63**:273.

Delarue, M., Poch, O., Tordo, N., Moras, D., and Argos, P., 1990, An attempt to unify the structure of polymerases, *Protein Eng.* **3**:461.

Delchambre, M., Gheysen, D., Thines, D., Thiriart, C., Jacobs, E., Verdin, E., Horth, M., Burny, A., and Bex, F., 1989, The Gag precursor of simian immunodeficiency virus assembles into virus-like particles, *EMBO J.* **8**:2653.

Desai, K., Loewenstein, P. M., and Green, M., 1991, Isolation of a cellular protein that binds to the human immunodeficiency virus Tat protein and can potentiate transactivation of the viral promoter, *Proc. Natl. Acad. Sci. USA* **88**:8875.

Desrosiers, R. C., 1992, HIV with multiple gene deletions as a live attenuated vaccine for AIDS, *AIDS Res. Hum. Retroviruses* **8**:411.

DeStefano, J. J., Buiser, R. G., Mallaber, L. M., Myers, T. W., Bambara, R. A., and Fay, P. J., 1991, Polymerization and RNase H activities of the reverse transcriptases from avian myeloblastosis, human immunodeficiency, and Moloney murine leukemia viruses are functionally uncoupled, *J. Biol. Chem.* **266**:7423.

DeZazzo, J. D., Scott, J. M., and Imperiale, M. J., 1992, Relative roles of signals upstream of AAUAAA and promoter proximity in regulation of human immunodeficiency virus type 1 mRNA 3′ end formation, *Mol. Cell. Biol.* **12**:5555.

Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Heaphy, S., Karn, J., Lowe, A. D., Singh, M., and Skinner, M. A., 1990, HIV-1 Tat protein stimulates transcription by binding to a U-rich bulge in the stem of the TAR RNA structure, *EMBO J.* **9**:4145.

Doms, R. W., Lamb, R. A., Rose, J. K., and Helenius, A., 1993, Folding and assembly of viral membrane proteins, *Virology* **193**:545.

Doolittle, R. F., Feng, D. F., Johnson, M. S., and McClure, M. A., 1989, Origin and evolutionary relationships of retroviruses, *Q. Rev. Biol.* **64**:1.

Dorfman, T., Mammano, F., Haseltine, W. A., and Gottlinger, H. G., 1994, Role of the matrix protein in the virion association of the human immunodeficiency virus type 1 envelope glycoprotein, *J. Virol.* **68**:1689.

Earl, P. L., Doms, R. W., and Moss, B., 1990, Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein, *Proc. Natl. Acad. Sci. USA* **87**:648.

Earl, P. L., Moss, B., and Doms, R. W., 1991, Folding, interaction with GRP78-BiP, assembly, and transport of the human immunodeficiency virus type 1 envelope protein, *J. Virol.* **65**:2047.

Eggermont, J., and Proudfoot, N. J., 1993, Poly(A) signals and transcriptional pause sites combine to prevent interference between RNA polymerase II promoters, *EMBO J.* **12**:2539.

Elder, J. H., Lerner, D. L., Hasselkus-Light, C. S., Fontenot, D. J., Hunter, E., Luciw, P. A., Montelaro, R. C., and Phillips, T. R., 1992, Distinct subsets of retroviruses encode dUTPase, *J. Virol.* **66**:1791.

Emerman, M., Guyader, M., Montagnier, L., Baltimore, D., and Muesing, M. A., 1987, The specificity of the human immunodeficiency virus type 2 trans-activator is different from that of human immunodeficiency virus type 1, *EMBO J.* **6**:3755.

Engelman, A., Mizuuchi, K., and Craigie, R., 1991, HIV-1 DNA integration: Mechanism of viral DNA cleavage and DNA strand transfer, *Cell* **67**:1211.

Ensoli, B., Garillari, G., Salahuddin, S. Z., Gallo, R. C., and Wong-Staal, F., 1990, Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients, *Nature* **3**:84.

Erice, A., and Balfour, H., Jr., 1994, Resistance of human immunodeficiency virus type 1 to antiretroviral agents: A review, *Clin. Infect. Dis.* **18**:149.

Fan, L., and Peden, K., 1992, Cell-free transmission of Vif mutants of HIV-1, *Virology* **190**:19.

Fankhauser, C., Izaurralde, E., Adachi, Y., Wingfield, P., and Laemmli, U., 1991, Specific complex of human immunodeficiency virus type 1 Rev and nucleolar B23 proteins: Dissociation by the Rev response element, *Mol. Cell. Biol.* **11**:2567.

Fauci, A. S., 1991, Mechanisms in human immunodeficiency virus (HIV) infection, *Ann. Intern. Med.* **114**:678.

Felber, B. K., and Pavlakis, G. N., 1993, Molecular biology of HIV-1: Positive and negative regulatory elements important for virus expression, *AIDS* **7**:S51.

Fisher, A. G., Ensoli, B., Ivanoff, I., Chamberlain, M., Petteway, S., Ratner, L., Gallo, R. C., and

Wong-Staal, F., 1987, The *sor* gene of HIV-1 is required for efficient virus transmission *in vitro*, *Science* **237**:888.

Franchini, G., Rusche, J. R., O'Keefe, T. J., and Wong-Staal, F., 1988, The human immunodeficiency virus type 2 (HIV-2) contains a novel gene encoding a 16-kDa protein associated with mature virions, *AIDS Res. Hum. Retroviruses* **4**:243.

Frankel, A. D., 1992a, Activation of HIV transcription by Tat, *Curr. Opin. Genet. Dev.* **2**:293.

Frankel, A. D., 1992b, Peptide models of the Tat-TAR protein–RNA interaction, *Protein Sci.* **1**:1539.

Frankel, A. D., Chen, L., Cotter, R. J., and Pabo, C. O., 1988, Dimerization of the Tat protein from human immunodeficiency virus: A cysteine-rich peptide mimics the normal metal-linked dimer interface, *Proc. Natl. Acad. Sci. USA* **85**:6297.

Fukasawa, M., Miura, T., Hagesawa, A., Morikawa, S., Tsujimoto, H., Miki, K., Kitamura, A. T., and Hayami, M., 1988, Sequence of simian immunodeficiency virus from African green monkey, a new member of the HIV/SIV group, *Nature* **333**:457.

Fultz, P. N., 1994, Simian T-lymphotropic virus type I, in: *The Retroviridae*, Vol. 3 (J. A. Levy, ed.), pp. 111–131, Plenum Press, New York.

Gabuzda, D. H., Lawrence, K., Langhoff, E., Terwilliger, E., Dorfman, T., Haseltine, W. A., and Sodroski, J., 1992, Role of Vif in replication of human immunodeficiency virus type 1 in $CD4^+$ lymphocytes, *J. Virol.* **66**:6489.

Gait, M. J., and Karn, J., 1993, RNA recognition by the human immunodeficiency virus Tat and Rev proteins, *Trends Biochem. Sci.* **18**:255.

Gallaher, W. R., Ball, J. R., Garry, R. F., Griffin, M. C., and Montelaro, R. C., 1989, A general model for the transmembrane proteins of HIV and other retroviruses, *AIDS Res. Hum. Retroviruses* **5**:431.

Gallo, R., Wong-Staal, F., Montagmer, L., Haseltine, W. A., and Yoshida, M., 1988, HIV–HTLV gene nomenclature, *Nature* **333**:504.

Garcia, J. V., and Miller, A. D., 1991, Serine phosphorylation independent downregulation of cell-surface CD4 by Nef, *Nature* **350**:508.

Garcia, J. V., Alfano, J., and Miller, A. D., 1993, The negative effect of human immunodeficiency virus type 1 Nef on cell surface CD4 expression is not species-specific and requires the cytoplasmic domain of CD4, *J. Virol.* **67**:1511.

Gardner, M. B., and Luciw, P. A., 1992, Simian retroviruses, in: *AIDS and Other Manifestations of HIV Infection* (G. P. Wormser, ed.), p. 127, Raven Press, New York.

Gardner, M. B., Endres, M., and Barry, P., 1994, The simian retroviruses: SIV and SRV, in: *The Retroviridae*, Vol. 3 (J. A. Levy, ed.), pp. 133–276, Plenum Press, New York.

Garrett, E. D., Tiley, L. S., and Cullen, B. R., 1991, Rev activates expression of the human immunodeficiency virus type 1 Vif and *vpr* gene products, *J. Virol.* **65**:1653.

Gelderblom, H. R., 1991, Assembly and morphology of HIV: Potential effect of structure on viral function, *AIDS* **5**:617.

Gelderblom, H. R., Ozel, M., and Pauli, G., 1989, Morphogenesis and morphology of HIV. Structure–function relations, *Arch. Virol.* **106**:1.

Gendelman, H. E., and Morahan, P. S., 1992, Macrophages in viral infections, in: *The Macrophage* (C. E. Lewis and J. O. McGee, eds), p. 1457, Oxford University Press/IRL Press, New York.

Geyer, H., Holsbach, C., Hunsmann, G., and Schneider, J., 1988, Carbohydrates of human immunodeficiency virus, *J. Biol. Chem.* **263**:11760.

Gheysen, D., Jacobs, E., De Foresta, F., Thiriart, C., Francotte, M., Thines, D., and De Wilde, M., 1989, Assembly and release of HIV-1 precursor Pr55 Gag virus-like particles from recombinant baculovirus-infected insect cells, *Cell* **59**:103.

Gibbs, J. S., and Desrosiers, R. C., 1993, Auxilliary proteins of the primate immunodeficiency viruses, in: *Human Retroviruses* (B. R. Cullen, ed.), p. 137, IRL Press, Oxford, England.

Goff, S. P., 1992, Genetics of retroviral integration, *Annu. Rev. Genet.* **26**:527.

Gorelick, R. J., Nigida, S. M., Bess, J. W., Arthur, L. O., Henderson, L. E., and Rein, A., 1990, Noninfectious human immunodeficiency virus type 1 mutants deficient in genomic RNA, *J. Virol.* **64**:3207.

Gorman, J. J., Corino, G. L., and Selleck, P. W., 1990, Comparison of the positions and efficiency of

cleavage activation of fusion protein precursors of virulent and avirulent strains of Newcastle disease virus: Insights into the specificities of activating proteases, *Virology* **177**:339.

Gottlinger, H. G., Sodroski, J. G., and Haseltine, W. A., 1989, Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1, *Proc. Natl. Acad. Sci. USA* **86**:5781.

Greene, W. C., 1991, The molecular biology of human immunodeficiency virus type 1 infection, *N. Engl. J. Med.* **324**:308.

Greenway, A. L., McPhee, D. A., Grgacic, E., Hewish, D., Lucantoni, A., Macreadie, I., and Azad, A., 1994, Nef 27, but not the Nef 25 isoform of human immunodeficiency virus-type 1 pNL4.3 down-regulates surface CD4 and IL-2R expression in peripheral blood mononuclear cells and transformed T cells, *Virology* **198**:245.

Grief, C., Hcokley, D. J., Fromholc, C. E., and Kitchin, P. A., 1989, The morphology of simian immunodeficiency virus as shown by negative staining electron microscopy, *J. Gen. Virol.* **70**:2215.

Grimalia, R. J., Fuller, B. A., Rennert, P. D., Nelson, M. B., Hammarskjold, M. L., Potts, B., Murray, M., Putney, S. D., and Gray, G., 1992, Mutations in the principal neutralization domain of the human immunodeficiency virus type 1 affect syncytium formation, virus infectivity, growth kinetics, and neutralization, *J. Virol.* **66**:1875.

Guy, B., Keiny, M.-P., and Rivière, Y., 1987, HIV F/3′-orf encodes a phosphorylated GTP-binding protein resembling an oncogene product, *Nature* **330**:266.

Guy, B., Rivière, Y., Dott, K., Regnault, A., and Kieny, M. P., 1990, Mutational analysis of the HIV nef protein, *Virology* **176**:413.

Guy, B., Geist, M., Dott, K., Spehner, D., Kieny, M.-P., and Lecocq, J.-P., 1991, A specific inhibitor of cysteine proteases impairs a Vif-dependent modification of human immunodeficiency virus type 1 Env protein, *J. Virol.* **65**:1325.

Guyader, M., Emerman, M., Sonigo, P., Clavel, F., Montagnier, L., and Alizon, M., 1987, Genome organization and transactivation of HIV-2, *Nature* **326**:662.

Harrison, G. P., and Lever, A. M. L., 1992, The human immunodeficiency virus type 1 packaging and major splice donor region have a conserved stable secondary structure, *J. Virol.* **66**:4144.

Haseltine, W. A., 1991, Molecular biology of the human immunodeficiency virus type 1, *FASEB J.* **5**:2349.

Hatfield, D. L., Levin, J. G., Rein, A., and Oroszlan, S., 1992, Translational suppression in retroviral gene expression, *Adv. Virus Res.* **41**:193.

Hattori, N., Michaels, F., Fargnoli, K., Marcon, L., Gallo, R. C., and Franchini, G., 1990, The human immunodeficiency virus type 2 *vpr* gene is essential for productive infection of human macrophages, *Proc. Natl. Acad. Sci. USA* **87**:8080.

Hattori, T., Koito, A., Takasuki, K., Kido, H., and Katunuma, N., 1989, Involvement of a tryptase-related cellular protease(s) in human immunodeficiency virus type 1 infection, *FEBS Lett.* **248**:48.

Hauber, J., Malim, M. H., and Cullen, B. R., 1989, Mutational analysis of the conserved basic domain of human immunodeficiency virus Tat protein, *J. Virol.* **63**:1181.

Heaphy, S., Finch, J. T., Gait, M. J., Karn, J., and Singh, M., 1991, Human immunodeficiency virus type 1 regulator of virion expression, Rev, forms nucleoprotein filaments after binding to a purine-rich "bubble" located within the rev-responsive region of viral mRNAs, *Proc. Natl. Acad. Sci. USA* **88**:7366.

Henderson, L. E., Sowder, R. C., Copeland, T. D., Benveniste, R. E., and Oroszlan, S., 1988, Isolation and characterization of a novel protein (X-ORF product) from SIV and HIV-2, *Science* **241**:199.

Henderson, L. E., Sowder, R. C., Copeland, T. D., Oroszlan, S., and Benveniste, R. E., 1990, Gag precursors of HIV and SIV are cleaved into six proteins found in the mature virions, *J. Med. Primatol.* **19**:411.

Henderson, L. E., Bowers, M. A., Sowder, R. C., Serabyn, S. A., Johnson, D. G., Bess, J. W., Arthur, L. O., Bryant, D. K., and Fenselau, C., 1992, Gag proteins of the highly replicative MN strain of human immunodeficiency virus type 1: Posttranslational modifications, proteolytic processing, and complete amino acid sequences, *J. Virol.* **66**:1856.

Herrmann, C. H., and Rice, A. P., 1993, Specific interaction of the human immunodeficiency virus Tat proteins with a cellular protein kinase, *Virology* **197**:601.

Hirsch, V. M., Edmonson, P., Murphey-Corb, M., Arbeille, B., Johnson, P. R., and Mullins, J. I., 1989, SIV adaptation to human cells, *Nature* **341**:573.

Hoglund, S., Ofverstedt, L.-G., Nilsson, A., Lundquist, P., Gelderblom, H., Ozel, M., and Skoglund, U., 1992, Spatial visualization of the maturing HIV-1 core and its linkage to the envelope, *AIDS Res. Hum. Retroviruses* **8**:1.

Hu, W., Vander Heyden, N., and Ratner, L., 1989, Analysis of the function of viral protein X (VPX) of HIV-2, *Virology* **173**:624.

Hunter, E., and Swanstrom, R., 1990, Retrovirus envelope glycoproteins, *Curr. Topics Microbiol. Immunol.* **157**:187.

Imperiale, M. J., and DeZazzo, J. D., 1991, Poly(A) site choice in retroelements: Déjà vu all over again? *New Biol.* **3**:531.

Jacks, T., 1990, Translational suppression in gene expression in retroviruses and retrotransposons, in: *Retroviruses–Strategies of Replication* (R. Swanstrom and P. K. Vogt, eds.), p. 93, Springer-Verlag, Berlin.

Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J., and Varmus, H. E., 1988, Characterization of ribosomal frameshifting in HIV-1 Gag-Pol expression, *Nature* **331**:280.

Jacobo-Molina, A., and Arnold, E., 1991, HIV reverse transcriptase structure–function relationships, *Biochemistry* **30**:6351.

Jacobo-Molina, A., Ding, J., Nanni, R. G., Clark, A., Jr., Lu, X., Tantillo, C., Williams, R. L., Kamer, G., Ferris, A. L., Clark, P., Hizi, A., Hughes, S. H., and Arnold, E., 1993, Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 Å resolution shows bent DNA, *Proc. Natl. Acad. Sci. USA* **90**:6320.

Jeang, K. T., Chun, R., Lin, N. H., Gatignol, A., Glabe, C. G., and Fan, H., 1993, *In vitro* and *in vivo* binding of human immunodeficiency virus type 1 Tat protein and Sp1 transcription factor, *J. Virol.* **67**:6224.

Jones, K. A., and Peterlin, B. M., 1994, Control of RNA initiation and elongation at the HIV-1 promoter, *Annu. Rev. Biochem.* **63**:717.

Jones, K. S., Coleman, J., Merkel, G. W., Laue, T. M., and Skalka, A. M., 1992, Retroviral integrase functions as a multimer and can turn over catalytically, *J. Biol. Chem.* **267**:16037.

Kan, N. C., Franchini, G., Wong-Staal, F., DuBois, G. C., Robey, W. G., Lautenberger, J. A., and Papas, T. S., 1986, Identification of HTLV-III/LAV sor gene product and detection of antibodies in human sera, *Science* **231**:1553.

Kao, S.-Y., Calman, A. F., Luciw, P. A., and Peterlin, B. M., 1987, Anti-termination of transcription within the long terminal repeat of HIV-1 by *tat* gene product, *Nature* **330**:489.

Kappes, J. C., Conway, J. A., Lee, S. W., Shaw, G. M., and Hahn, B. H., 1991, Human immunodeficiency virus type 2 Vpx protein augments viral infectivity, *Virology* **184**:197.

Kappes, J. C., Parkin, J. S., Conway, J. A., Kim, J., Brouillette, C. G., Shaw, G. M., and Hahn, B. H., 1993, Intracellular transport and virion incorporation of px requires interaction with other virus type-specific components, *Virology* **193**:222.

Karn, J., 1991, Control of human immunodeficiency virus replication by the *tat*, *rev*, *nef*, and *protease* genes, *Curr. Opin. Immunol.* **3**:526.

Karn, J., and Graeble, M. A., 1992, New insights into the mechanism of HIV-1 *trans*-activation, *Trends Genet.* **8**:365.

Karpel, R. L., Henderson, L. E., and Oroszlan, S., 1987, Interactions of retroviral structural proteins with single-stranded nucleic acids, *J. Biol. Chem.* **262**:4961.

Kashanchi, F., Piras, G., Radonovich, M. F., Duvall, J. F., Fattaey, A., Chiang, C. M., Roeder, R. G., and Brady, J. N., 1994, Direct interaction of human TFIID with the HIV-1 transactivator Tat, *Nature* **367**:295.

Kato, H., Sumimoto, H., Pognonec, P., Chen, C. H., Rosen, C. A., and Roeder, R. G., 1992, HIV-1 Tat acts as a processivity factor *in vitro* in conjunction with cellular elongation factors, *Genes Dev.* **6**:655.

Katz, R. A., and Skalka, A. M., 1990, Generation of diversity in retroviruses, *Annu. Rev. Genet.* **24**:409.

Kestler, H. W., Ringler, D. J., Mori, K., Panicall, D., Sehgal, P., Daniel, M., and Desrosiers, R. C., 1991, Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS, *Cell* **65:**651.

Kienzle, N., Freund, J., Kalbitzer, H. R., and Mueller-Lantzsch, N., 1993, Oligomerization of the Nef protein from human immunodeficiency virus (HIV) type 1, *Eur. J. Biochem.* **214:**451.

Kim, S., Byrn, R., Groopman, J., and Baltimore, D., 1989, Temporal aspects of DNA and RNA synthesis during human immunodeficiency virus infection: Evidence for differential gene expression, *J. Virol.* **63:**3708.

Kjems, J., Frankel, A. D., and Sharp, P. A., 1991, Specific regulation of mRNA splicing *in vitro* by a peptide from HIV-1 Rev, *Cell* **67:**169.

Klasse, P. J., Moore, J. P., and Jameson, B. A., 1993, The interplay of the HIV-1 envelope complex, gp120 and gp41 with CD4, in: *HIV: Molecular Organization, Pathogenicity and Treatment* (W. J. W. Morrow and N. L. Haigwood, eds.), p. 241, Elsevier, Amsterdam.

Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guétard, D., Hercend, T., Gluckman, J. C., and Montagnier, L., 1984, T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV, *Nature* **312:**767.

Klenerman, P., Rowland-Jones, S., McAdam, S., Edwards, J., Daenke, S., Lalloo, D., Koppe, B., Rosenberg, W., Boyd, D., Edwards, A., Giangrande, P., Philips, R. E., and McMichael, A. J., 1994, Cytotoxic T-cell activity antagonized by naturally occurring HIV-1 Gag variants, *Nature* **369:**403.

Klimkait, T., Strebel, K., Hoggan, M. D., Martin, M. A., and Orenstein, J. M., 1990, The human immunodeficiency virus type 1-specific protein Vpu is required for efficient virus maturation and release, *J. Virol.* **64:**621.

Kodama, T., Wooley, D. P., Naidu, Y. M., Kestler III, H. W., Daniel, M. D., Li, Y., and Desrosiers, R. C., 1989, Significance of premature stop codons in *env* of simian immunodeficiency virus, *J. Virol.* **63:**4709.

Koga, Y., Lindstrom, E., Fenyo, A. M., Wigzell, H., and Mak, T. W., 1988, High levels of heterodisperse RNAs accumulate in T cells infected with human immunodeficiency virus and in normal thymocytes, *Proc. Natl. Acad. Sci. USA* **85:**4521.

Kohlstaedt, L. A., Wang, J. M., Friedman, J. M., Rice, P. A., and Steitz, T. A., 1992, Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor, *Science* **256:**1783.

LaFemina, R. L., Callahan, P. L., and Cordingley, M. G., 1991, Substrate specificity of recominant human immunodeficiency virus integrase protein, *J. Virol.* **65:**5624.

Lang, S., Weeger, M., Stahl-Hennig, C., Coulibaly, C., Hunsmann, G., Muller, J., Muller-Hermelink, H., Fuchs, D., Wachter, H., Daniel, M. M., Desrosiers, R. C., and Fleckenstein, B., 1993, Importance of Vpr for infection of rhesus monkeys with simian immunodeficiency virus, *J. Virol.* **67:**902.

Larder, B. A., and Kemp, S. D., 1989, Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT), *Science* **246:**1155.

LaRosa, G. J., Davide, J. P., Weinhold, K., Waterbury, J. A., Profy, A. T., Lewis, J. A., Langlois, A., Dreesman, G. R., Boswell, R. N., Shadduck, P., Holley, L. H., Karplus, M., Bolognesi, D. P., Matthews, T. J., Emini, E. A., and Putney, S. D., 1990, Conserved sequence and structural elements in the HIV-1 principal neutralizing determinant, *Science* **249:**932.

Lasky, L. A., Nakamura, G., Smith, D. H., Fennie, C., Shimasaki, C., Patzer, E., Berman, P., Gregory, T., and Capon, D. J., 1987, Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor, *Cell* **50:**975.

Le, S.-Y., Malim, M. H., Cullen, B. R., and Maizel, J. V., 1990, A highly conserved RNA folding region coincident with the Rev response element of primate immunodeficiency viruses, *Nucleic Acids Res.* **18:**1613.

Lee, T. H., Coligan, J. E., Allan, J. S., McLane, M. F., Groopman, J. E., and Essex, M., 1986, A new HTLV-III/Lav protein encoded by a gene found in cytopathic retroviruses, *Science* **231:**1546.

LeGrice, S. F. J., 1993, Human immunodeficiency virus reverse transcriptase, in: *Reverse Transcriptase* (A. M. Skalka and S. P. Goff, eds.), p. 163, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Leonard, C. K., Spellman, M. W., Riddle, L., Harris, R. J., Thomas, J. N., and Gregory, T. J., 1990, Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells, *J. Biol. Chem.* **265**:10373.

Lever, A. M. L., Richardson, J. H., and Harrison, G. P., 1991, Retroviral RNA packaging, *Biochem. Soc. Trans.* **19**:963.

Levy, D. N., Fernandes, L. S., Williams, W. V., and Weiner, D. B., 1993, Induction of cell differentiation by human immunodeficiency virus 1 Vpr, *Cell* **72**:541.

Levy, J. A., 1993, Pathogenesis of human immunodeficiency virus infection, *Microbiol. Rev.* **57**:183.

Lewis, N., Williams, J., Rekosh, D., and Hammarskjold, M.-L., 1990, Identification of a *cis*-acting element in human immunodeficiency virus type 2 (HIV-2) that is responsive to the HIV-1 Rev and human T-cell leukemia virus types 1 and 2 Rex proteins, *J. Virol.* **64**:1690.

Lindemann, D., Wilhelm, R., Renard, P., Althage, A., Zinkernagel, R., and Mous, J., 1994, Severe immunodeficiency associated with a human immunodeficiency virus 1 Nef/3′-long terminal repeat transgene, *J. Exp. Med.* **179**:797.

Linial, M. L., and Miller, A. D., 1990, Retroviral RNA packaging: Sequence requirements and implications, *Curr. Topics Microbiol. Immunol.* **157**:125.

Lu, X., Welsh, T., and Peterlin, B. M., 1993, The human immunodeficiency virus type 1 long terminal repeat specifies two different transcription complexes, only one of which is regulated by Tat, *J. Virol.* **67**:1752.

Lu, Y. L., Spearman, P., and Ratner, L., 1993, Human immunodeficiency virus type 1 viral protein R localization in infected cells and virions, *J. Virol.* **67**:6542.

Luban, J., Bossolt, K. L., Franke, E. K., Kalpana, G. V., and Goff, S. P., 1993, Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B, *Cell* **73**:1067.

Luciw, P. A., and Leung, N. J., 1992, Mechanisms of retrovirus replication, in: *The Retroviridae*, Vol. 1 (J. A. Levy, ed.), pp. 159–298, Plenum Press, New York.

Luciw, P. A., and Shacklett, B. L., 1993, Molecular biology of the human and simian immunodeficiency viruses, in: *HIV: Molecular Organization, Pathogenicity and Treatment* (W. J. W. Morrow and N. L. Haigwood, eds.), p. 123, Elsevier, Amsterdam.

Luciw, P. A., Shaw, K. E. S., Unger, R., Planelles, V., Stout, M. W., Lackner, J. E., Pratt-Lowe, E., Leung, N. J., Banapour, B., and Marthas, M. L., 1992, Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac), *AIDS Res. Hum. Retroviruses* **8**:395.

Luo, Y., Madore, S. J., Parslow, T. G., Cullen, B. R., and Peterlin, B. M., 1993, Functional analysis of interactions between Tat and the *trans*-activation response element of human immunodeficiency virus type 1 in cells, *J. Virol.* **67**:5617.

Luo, Y., Yu, H., and Peterlin, B. M., 1994, Cellular protein modulates effects of human immunodeficiency virus type 1 Rev, *J. Virol.* **68**:3850.

Luria, S., Chambers, I., and Berg, P., 1991, Expression of the type 1 human immunodeficiency virus Nef protein in T cells prevents antigen receptor-mediated induction of interleukin-2 mRNA, *Proc. Natl. Acad. Sci. USA* **88**:5326.

Mak, J., Jiang, M., Wainberg, M. A., Hammarskjold, M. L., Rekosh, D., and Kleiman, L., 1994, Role of $Pr160^{gag\text{-}pol}$ in mediating the selective incorporation of tRNA(Lys) into human immunodeficiency virus type 1 particles, *J. Virol.* **68**:2065.

Malim, M. H., and Cullen, B. R., 1991, HIV-1 structural gene expression requires the binding of multiple Rev monomers to the viral RRE: Implications for HIV-1 latency, *Cell* **65**:241.

Malim, M. H., and Cullen, B. R., 1993, Rev and the fate of pre-mRNA in the nucleus: Implications for the regulation of RNA processing in eukaryotes, *Mol. Cell. Biol.* **13**:6180.

Malim, M. H., Tiley, L. S., McCarn, D. F., Rusche, J. R., Hauber, J., and Cullen, B. R., 1990, HIV-1 structural gene expression requires binding of the Rev *trans*-activator to its RNA target sequence, *Cell* **60**:675.

Marcon, L., Michaels, F., Hattori, N., Fargnoli, K., Gallo, R. C., and Franchini, G., 1991, Dispensable role of the human immunodeficiency virus type 2 Vpx protein in viral replication, *J. Virol.* **65**:3938.

Marquet, R., Baudin, F., Gabus, C., Darlix, J. L., Mougel, M., Ehresman, C., and Ehresman, B., 1991,

Dimerization of human immunodeficiency virus (type 1) RNA: Stimulation by cations and possible mechanism, *Nucleic Acids Res.* **19:**2349.

Marquet, R., Paillart, J. C., Skripkin, E., Ehresman, C., and Ehresmann, B., 1994, Dimerization of human immunodeficiency virus type 1 RNA involves sequences located upstream of the splice donor site, *Nucleic Acids Res.* **22:**145.

Marx, P. A., Munn, R. J., and Joy, K. I., 1988, Computer emulation of thin section electron microscopy predicts an envelope-associated icosadeltahedral capsid for human immunodeficiency virus, *Lab Invest.* **58:**112.

Matsuda, Z., Chou, M. J., Matsuda, M., Huang, J. H., Chen, Y. M., Redfield, R., Mayer, K., Essex, M., and Lee, T. H., 1988, Human immunodeficiency virus type 1 has an additional coding sequence in the central region of the genome, *Proc. Natl. Acad. Sci. USA* **85:**6968.

McClure, M. O., Sommerfelt, M. A., Marsh, M., and Weiss, R. A., 1990, The pH independence of mammalian retrovirus infection, *J. Gen. Virol.* **71:**767.

McCune, J. M., Rabin, L. B., Feinberg, M. B., Lieberman, M., Kosek, J. C., Reyes, G. R., and Weissman, I. L., 1988, Endoproteolytic cleavage of gp160 is required for activation of human immunodeficiency virus, *Cell* **53:**55.

McDougal, J. S., Nicholson, J. K. A., Cross, G. D., Cort, S. P., Kennedy, M. S., and Mawle, A. C., 1986, Binding of the human retrovirus HTLV-III/ARV/HIV to the CD4 (T4) molecule: Conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry, *J. Immunol.* **137:**2937.

Milich, L., Margolin, B., and Swanstrom, R., 1993, V3 loop of the human immunodeficiency virus type 1 Env protein: interpreting sequence variability, *J. Virol.* **67:**5623.

Miller, M., Schneider, J., Sathyanrayana, B. K., Toth, M. Y., Marshall G. R., Clawson, L., Selk, L., Kent, S. B. H., and Wlodawer, A., 1989, Structure of complex of synthetic HIV-1 protease with a substrate-based inhibitor at 2.3 Å resolution, *Science* **246:**1149.

Miller, M. D., Warmerdam, M. T., Gaston, I., Greene, W. C., and Feinberg, M. B., 1994, The human immunodeficiency virus-1 nef gene product: A positive factor for viral infection and replication in primary lymphocytes and macrophages, *J. Exp. Med.* **179:**101.

Mitsuya, H., Yarochan, R., and Broder, S., 1990, Molecular targets for AIDS therapy, *Science* **249:** 1533.

Mizuochi, T., Matthews, T. J., Kato, M., Hamako, J., Titani, K., Solomon, J., and Feizi, T., 1990, Diversity of oligosaccharide structure on the envelope glycoprotein gp120 of human immunodeficiency virus 1 from the lymphoblastoid cell line H9. Presence of complex-type oligosaccharides with bisecting *N*-acetylglucosamine residues, *J. Biol. Chem.* **265:**8519.

Mizuuchi, K., and Adzuma, K., 1991, Inversion of the phosphate chirality at the site of Mu DNA strand transfer: Evidence for a one-step transesterification mechanism, *Cell* **66:**129.

Modesti, N., Garcia, J., DeBouck, C., Peterlin, B. M., and Gaynor, R., 1991, Transdominant Tat mutants with alterations in the basic domain inhibit HIV-1 gene expression, *New Biol.* **3:**759.

Moore, J. P., 1990, Simple methods for monitoring HIV-1 and HIV-2 gp120 binding to sCD4 by ELISA: HIV-2 has a 25-fold lower affinity than HIV-1 for sCD4, *AIDS* **3:**297.

Moore, J. P., and Nara, P. L., 1991, The role of the V3 loop of gp120 in HIV infection, *AIDS* **5:**S21.

Moore, J. P., Sattentau, Q. J., Wyatt, R., and Sodroski, J., 1994, Probing the structure of the human immunodeficiency virus surface glycoprotein gp120 with a panel of monoclonal antibodies, *J. Virol.* **68:**469.

Morrow, C. D., Park, J., and Wakefield, J. K., 1994, Viral gene products and replication of the human immunodeficiency type 1 virus, *Am. J. Pathol.* **266:**C1135.

Muesing, M. A., Smith, D. H., and Capon, D. J., 1987, Regulation of mRNA accumulation by a human immunodeficiency virus *trans*-activator protein, *Cell* **48:**691.

Mujeeb, A., Bishop, K., Peterlin, B. M., Turck, C., Parslow, T. G., and Janus, T. L., 1994, NMR structure of a biologically active peptide containing the RNA-binding domain of HIV-1 Tat, *Proc. Natl. Acad. Sci. USA* **91:**8248.

Myers, G., Berzofsky, J. A., Korber, B., Smith, R. F., and Pavlakis, G. N., 1993, *Human Retroviruses and AIDS 1993: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences*, Theoretical Biology and Biophysics Group T-10, Los Alamos National Library, Los Alamos, NM.

Nabel, G. J., 1993, The role of cellular transcription factors in the regulation of human immunodefi-

ciency virus gene expression, in: *Human Retroviruses* (B. R. Cullen, ed.), p. 49, IRL Press, Oxford, England.

Navia, M. A., and McKeever, B. M., 1990, A role for the aspartyl protease from the human immunodeficiency virus type 1 (HIV-1) in the orchestration of virus assembly, *Ann. NY Acad. Sci.* **616:**73.

Navia, M. A., Fitzgerald, P. M. D., and McKeever, B. M., 1989, Three dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1, *Nature* **337:**615.

Nelbock, P., Dillon, R., Perkins, A., and Rosen, C. A., 1990, A cDNA for a protein that interacts with the human immunodeficiency virus tat transactivator, *Science* **248:**1650.

Neuzil, K. M., 1994, Pharmacologic therapy for human immunodeficiency virus infection: A review, *Am. J. Med. Sci.* **307:**368.

Niederman, T. M., J., Thielan, G. J., and Ratner, L., 1989, Human immunodeficiency virus type 1 negative factor is a transcriptional silencer, *Proc. Natl. Acad. Sci. USA* **86:**1128.

Niederman, T. M. J., Hu, W., and Ratner, L., 1991, Simian immunodeficiency virus negative factor suppresses the level of viral mRNA in COS cells, *J. Virol.* **65:**3538.

Niedrig, M., Rabanus, J.-P., L'Age-Stehr, J., Gelderblom, H. R., and Pauli, G., 1988, Monoclonal antibodies directed against human immunodeficiency virus (HIV) Gag proteins with specificity for conserved epitopes in HIV-1, HIV-2, and simian immunodeficiency virus, *J. Gen. Virol.* **69:** 2109.

Oberste, M. S., and Gonda, M. A., 1992, Conservation of amino-acid sequence motifs in lentivirus Vif proteins, *Virus Genes* **6:**95.

Ogawa, K., Shibata, R., Kiyomasu, T., Higuchi, I., Kishida, Y., Ishimoto, A., and Adachi, A., 1989, Mutational analysis of the human immunodeficiency virus vpr open reading frame, *J. Virol.* **63:**4110.

Olsen, H. S., Cochrane, A. W., Dillon, P. J., Nalin, C. M., and Rosen, C. A., 1990, Interaction of the human immunodeficiency virus type 1 Rev protein with a structured region in *env* mRNA is dependent on multimer formation mediated through a basic stretch of amino acids, *Genes Dev.* **4:**1357.

Olsen, H. S., Beidas, S., Dillon, P., Rosen, C. A., and Cochrane, A. W., 1991, Mutational analysis of the HIV-1 Rev protein and its target sequence, the Rev response element, *J. AIDS* **4:**558.

Oroszlan, S., and Luftig, R. B., 1990, Retroviral proteinases, in: *Retroviruses—Strategies of Replication* (R. Swanstrom and P. K. Vogt, eds.), p. 153, Springer-Verlag, Berlin.

Owens, R., Dubay, J. W., Hunter, E., and Compans, R. W., 1991, Human immunodeficiency virus envelope protein determines the site of virus release in polarized epithelial cells, *Proc. Natl. Acad. Sci. USA* **88:**3987.

Pantaleo, G., Graziosi, C., and Fauci, A. S., 1993, The immunopathogenesis of human immunodeficiency virus infection, *N. Engl. J. Med.* **328:**327.

Parslow, T. G., 1993, Post-transcriptional regulation of human retroviral gene expression, in: *Human Retroviruses* (B. R. Cullen, ed.), p. 101, IRL Press, Oxford, England.

Pathak, V.K., and Temin, H. M., 1990, Broad spectrum of *in vivo* forward mutations, hypermutations, and mutational hot-spots in a retroviral shuttle vector after a single replication cycle: Substitutions, frameshifts, and hypermutations, *Proc. Natl. Acad. Sci. USA* **87:**6019.

Pavlakis, G. N., Schwartz, S., D'Agostino, D., and Felber, B., 1992, Structure, splicing, and regulation of expression of HIV-1: A model for the general organization of lentiviruses and other complex retroviruses, in: *AIDS Research Reviews* (W. C. Koff, R. C. Kennedy, and F. Wong-Staal, eds.), p. 41, Marcel Dekker, New York.

Paxton, W., Connor, R. I., and Landau, N. R., 1993, Incorporation of Vpr into human immunodeficiency virus type 1 virions: Requirement for the p6 region of Gag and mutational analysis, *J. Virol.* **67:**7229.

Peliska, J. A., and Benkovic, S. J., 1992, Mechanism of DNA strand transfer reactions catalyzed by HIV-1 reverse transcriptase, *Science* **258:**1112.

Peterlin, B. M., Adams, M., Alonso, A., Baur, A., Ghosh, S., Lu, X., and Luo, Y., 1993, Tat transactivator, in: *Human Retroviruses* (B. R. Cullen, ed.), p. 75, IRL Press, Oxford, England.

Pettit, S. C., Simsic, J., Loeb, D. D., Everitt, L., Hutchison, C. A., 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.

Phillips, R. E., Rowland-Jones, S., Nixon, D. F., Gotch, F. M., Edwards, J. P., Ogunlesi, A. O., Elvin, J. G., Rothbard, J. A., Bangham, C. R., Rizza, C. R., and McMichael, A. J., 1991, Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition, *Nature* **354:**453.

Pomerantz, R. J., Trono, D., Feinberg, M. B., and Baltimore, D., 1990, Cells nonproductively infected with HIV-1 exhibit an aberrant pattern of viral RNA expression: A molecular model for latency, *Cell* **61:**1271.

Preston, B. D., Poiez, B. J., and Loeb, L., 1988, Fidelity of HIV-1 reverse transcriptase, *Science* **242:** 1168.

Prongay, A. J., Smith, T. J., Rossman, M. G., Ehrlich, L. S., Carter, C. A., and McClure, J., 1990, Preparation and crystallization of a human immunodeficiency virus p24-Fab complex, *Proc. Natl. Acad. Sci. USA* **87:**9980.

Rappaport, J., Lee, S.-J., Khalili, K., and Wong-Staal, F., 1989, The acidic amino-terminal region of the HIV-1 Tat protein constitutes an essential activating domain, *New Biol.* **1:**101.

Robert-Guroff, M., Popovic, M., Gartner, S., Markham, P., Gallo, R. C., and Reitz, M. S., 1990, Structure and expression of tat-, rev-, and nef-specific transcripts of human immunodeficiency virus type 1 in infected lymphocytes and macrophages, *J. Virol.* **64:**3391.

Roberts, J. D., Bebenek, K., and Kunkel, T. A., 1988, The accuracy of reverse transcriptase from HIV-1, *Science* **242:**1171.

Roberts, J. D., Preston, B. D., Johnston, L. A., Soni, A., Loeb, L. A., and Kunkel, T. A., 1989, Fidelity of two retroviral reverse transcriptases during DNA-dependent DNA synthesis *in vitro*, *Mol. Cell. Biol.* **9:**469.

Robey, W. G., Safai, B., Oroszlan, S., Arthur, L. O., Gonda, M. A., Gallo, R. C., and Fischinger, P. J., 1985, Characterization of envelope and core structural gene products of HTVL-III with sera from AIDS patients, *Science* **228:**593.

Rosen, C. A., and Dillon, P. J., 1991, Regulatory proteins of the human immunodeficiency viruses, in: *AIDS Research Reviews* (W. C. Koff, R. C. Kennedy, and F. Wong-Staal, eds.), p. 3, Marcel Dekker, New York.

Rosen, C. A., Sodroski, J. G., and Haseltine, W. A., 1985, The location of *cis*-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat, *Cell* **41:**813.

Roy, S., Delling, U., Chen, C.-H., Rosen, C. A., and Sonenberg, N., 1990, A bulge structure in HIV-1 TAR RNA is required for Tat binding and Tat-mediated trans-activation, *Genes Dev.* **4:**1365.

Ruhl, M., Himmelspach, M., Bahr, G. M., Hammerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Farrington, G. K., Probst, H., Bevec, D., and Hauber, J., 1993, Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating *trans*-activation, *J. Cell. Biol.* **123:**1309.

Ryser, H. J., Levy, E. M., Mandel, R., and DiSciullo, G. J., 1994, Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulfide interchange upon virus-receptor interaction, *Proc. Natl. Acad. Sci. USA* **91:**4559.

Sakai, H., Siomi, H., Shida, H., Shibata, R., Kiyomasu, T., and Adachi, A., 1990, Functional comparison of transactivation by human retrovirus *rev* and *rex* genes, *J. Virol.* **64:**5833.

Sakai, H., Shibata, R., Sakuragi, J.-I., Sakuragi, S., Kawamura, M., and Adachi, A., 1993, Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles, *J. Virol.* **67:**1663.

Samuel, K. P., Hodge, D. R., Chen, Y.-M., and Papas, T., 1991, Nef proteins of the human immunodeficiency viruses (HIV-1 and HIV-2) and simian immunodeficiency virus (SIV) are structurally similar to leucine zipper transcriptional activation factors, *AIDS Res. Hum. Retroviruses* **7:**697.

Sato, A., Igarashi, H., Adachi, A., and Hayami, M., 1990, Identification and localization of *vpr* gene product of human immunodeficiency virus type 1, *Virus Genes* **4:**303.

Sawai, E. T., Baur, A., Struble, H., Peterlin, B. M., Levy, J. A., and Cheng-Mayer, C., 1994, Human immunodeficiency virus type 1 Nef associates with a cellular serine kinase in T lymphocytes, *Proc. Natl. Acad. Sci. USA* **91:**1539.

Schawaller, M., Smith, G. E., Skehel, J. J., and Wiley, D. C., 1989, Studies with cross-linking reagents on the oligomeric structure of the Env glycoprotein of HIV, *Virology* **172:**367.

Schuitemaker, H., Koot, M. Kootstra, N. A., Dercksen, M. W., de Goede, R. E., van Steenwijk, R. P., Lange, J. M., Shattenkerk, J. K., Miedema, F., and Tersmette, M., 1992, Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: Progression of disease is associated with a shift from monocytotrophic to T-cell-tropic virus populations, *J. Virol.* **66:**1354.

Schultz, A. M., Henderson, L. E., and Oroszlan, S., 1988, Fatty acylation of proteins, *Annu. Rev. Cell Biol.* **4:**611.

Schulz, T. F., Reeves, J. D., Hoad, J. G., Tailor, C., Stephens, P., Clements, G., Ortlepp, S., Page, K. A., Moore, J. P., and Weiss, R. A., 1993, Effect of mutations in the V3 loop on HIV-1 gp120 on infectivity and susceptibility to proteolytic cleavage, *AIDS Res. Hum. Retroviruses* **9:**159.

Schwartz, S., Felber, B. K., Benko, D. M., Fenyo, E. M., and Pavlakis, G. M., 1990, Cloning and functional analysis of multiply spliced mRNA species of human immunodeficiency virus type 1, *J. Virol.* **64:**2519.

Schwartz, S., Felber, B. K., and Pavlakis, G. N., 1991, Expression of human immunodeficiency virus type 1 *vif* and *vpr* mRNAs is Rev-dependent and regulated by splicing, *Virology* **183:**677.

Sheldon, M., Ratnasbapathy, R., and Hernandez, N., 1993, Characterization of the inducer of short transcripts, a human immunodeficiency virus type 1 transcriptional element that activates the synthesis of short RNAs, *Mol. Cell. Biol.* **13:**1251.

Sherman, P. A., and Fyfe, J. A., 1990, Human immunodeficiency virus integration protein expressed in *Escherichia coli* possesses selective DNA cleaving ability, *Proc. Natl. Acad. Sci. USA* **87:** 5119.

Sherman, P., Dickson, M. L., and Fyfe, J. A., 1992, Human immunodeficiency virus type 1 integration protein: DNA sequence requirements for cleaving and joining reactions, *J. Virol.* **66:** 3693.

Shibata, R., Miura, T., Hayami, M., Ogawa, K., Sakai, H., Kiyomasu, T., Ishimoto, A., and Adachi, A., 1990, Mutational analysis of the human immunodeficiency virus type 2 (HIV-2) genome in relation to HIV-1 and simian immunodeficiency virus SIV_{agm}, *J. Virol.* **64:**742.

Shugars, D. C., Smith, M. S., Glueck, D. H., Nantermet, P. V., Seillier-Moiseiwitsch, F., and Swanstrom, R., 1993, Analysis of human immunodeficiency virus type 1 nef gene sequences present *in vivo*, *J. Virol.* **67:**4639.

Sinangil, F., Loyter, A., and Volsky, D. J., 1988, Quantitative measurement of fusion between human immunodeficiency virus and cultured cells using membrane fluorescence dequenching, *FEBS Lett.* **239:**88.

Skalka, A. M., 1989, Retroviral proteases: First glimpses at the anatomy of a processing machine, *Cell* **56:**911.

Skalka, A. M., and Goff, S. P., 1993, *Reverse Transcriptase*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Smith, A. J., Srinivasakumar, N., Hammarskjold, M. L., and Rekosh, D., 1993, Requirements for incorporation of $pr160^{gag\text{-}pol}$ from human immunodeficiency virus type 1 into virus-like particles, *J. Virol.* **67:**2266.

Sodroski, J. G., Goh, W. C., Rosen, C. A., Tartar, A., Portetelle, D., Burny, A., and Haseltine, W. A., 1986, Replication and cytopathic potential of HTLV-III/LAV with *sor* gene deletions, *Science* **231:**1549.

Sova, P., and Volsky, D. J., 1993, Efficiency of viral DNA synthesis during infection of permissive and nonpermissive cells with *vif*-negative human immunodeficiency virus type 1, *J. Virol.* **67:** 6322.

Spina, C. A., Kwoh, T. J., Chowers, M. Y., Guatelli, J. C., and Richman, D. D., 1994, The importance of nef in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes, *J. Exp. Med.* **179:**115.

St. Clair, M. H., Martin, J. L., Tudor-Williams, G., Bach, M. C., Vavro, C. L., King, D. M., Kelam, P., Kemp, S. D., and Larder, B. A., 1991, Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase, *Science* **253:**1557.

Stein, B. S., and Engelman, E. G.,1990, Intracellular processing of the gp160 HIV-1 envelope precursor. Endoproteolytic cleavage occurs in a *cis* or medial component of the Golgi complex, *J. Biol. Chem.* **265:**2640.

Stein, B. S., Gowda, S. D., Lifson, J. D., Penhallow, R. C., Bensch, K. G., and Engleman, E. G., 1987, pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane, *Cell* **49:**659.

Strebel, K., Daugherty, D., Cohen, D., Folks, T., and Martin, M., 1987, The HIV "A" (sor) gene product is essential for virus infectivity, *Nature* **328:**728.

Strebel, K., Klimkait, T., and Martin, M. A., 1988, A novel gene of HIV-1 vpu, and its 16 kilodalton product, *Science* **241:**1221.

Strebel, K., Klimkait, T., Maldarelli, F., and Martin, M. A., 1989, Molecular and biochemical analyses of human immunodeficiency virus type 1 vpu protein, *J. Virol.* **63:**3784.

Subramanian, T., Kuppuswamy, M., Venkatesh, L., Srinivasan, A., and Chinnadurai, G., 1990, Functional substitution of the basic domain of the HIV-1 *trans*-activator, Tat, with the basic domain of the functionally heterologous Rev, *Virology* **176:**178.

Surovoy, A., Dannuli, J., Moelling, K., and Jung, G., 1993, Conformational and nucleic acid binding studies on the synthetic nucleocapsid protein of HIV-1, *J. Mol. Biol.* **229:**94.

Swanstrom, R., Kaplan, A. H., and Manchester, M., 1990, The aspartic proteinase of HIV-1, in: *Retrovirus Genome Organization and Gene Expression* (I. S. Y. Chen, ed.), p. 175, Saunders, Philadelphia.

Tan, R., Chen, L., Buettner, J. A., Hudson, D., and Frankel, A. D., 1993, RNA recognition by an isolated alpha helix, *Cell* **73:**1031.

Terwilliger, E., 1992, The accessory gene functions of the primate immunodeficiency viruses, in: *AIDS Research Reviews* (W. C. Koff, R. C. Kennedy, and F. Wong-Staal, eds.), p. 3, Marcel Dekker, New York.

Toh, H., Ono, M., Saigo, K., and Miyata, T., 1985, Retroviral protease-like sequence in the yeast transposon Tyl, *Nature* **315:**691.

Tong-Starksen, S. E., Baur, A., Lu, X. B., Peck, E., and Peterlin, B. M., 1993, Second exon of Tat of HIV-2 is required for optimal *trans*-activation of HIV-1 and HIV-2 LTRs, *Virology* **195:**826.

Tristem, M., Marshall, C., Karpas, A., Petrik, J., and Hill, F., 1990, Origin of vpx in lentiviruses, *Nature* **347:**341.

Tristem, M., Marshall, C., Karpas, A., and Hill, F., 1992, Evolution of the primate lentiviruses: Evidence from *vpx* and *vpr*, *EMBO J.* **11:**3405.

Trono, D., 1992, Partial reverse transcripts in virions from human immunodeficiency and murine leukemia viruses, *J. Virol.* **66:**4893.

Trono, D., Feinberg, M. B., and Baltimore, D., 1989, Gag mutants can dominantly interfere with the replication of the wild-type virus, *Cell* **59:**113.

Vaishnav, Y. N., and Wong-Staal, F., 1991, The biochemistry of AIDS, *Annu. Rev. Biochem.* **60:**577.

Vaishnav, Y. N., Vaishnav, M., and Wong-Staal, F., 1991, Identification and characterization of a nuclear factor that specifically binds to the Rev-response element (RRE) of human immunodeficiency virus type 1 (HIV-1), *New Biol.* **3:**142.

Varmus, H., and Brown, P., 1989, Retroviruses, in: *Mobile DNA* (D. E. Berg and M. M. Howe, eds.), p. 53, American Society for Microbiology, Washington, DC.

Vickers, T. A., and Ecker, D. J., 1992, Enhancement of ribosomal frameshifting by oligonucleotides targeted to the HIV *gag-pol* region, *Nucleic Acids Res.* **20:**3845.

Vincent, K. A., Ellison, V., Chow, S. A., and Brown, P. O., 1993, Characterization of human immunodeficiency virus type 1 integrase expressed in *Escherichia coli* and analysis of variants with amino-terminal mutations, *J. Virol.* **67:**425.

Vink, C., van Gent, D. C., Elgersma, Y., and Plasterk, R. H. A., 1991a, Human immunodeficiency virus integrase protein requires a subterminal position of its viral DNA recognition sequence for efficient cleavage, *J. Virol.* **65:**4636.

Vink, C., Yeheskiely, E., van der Marel, G. A., van Boom, J. H., and Plasterk, R. H. A., 1991b, Site-specific hydrolysis and alcoholysis of human immunodeficiency virus DNA termini mediated by the viral integrase protein, *Nucleic Acids Res.* **19:**6691.

Vogel, J., Hinrichs, S. H., Reynolds, R. K., Luciw, P. A., and G. J., 1988, The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice, *Nature* **335:**606.

von Schwedler, U., Song, J., Aiken, C., and Trono, D., 1993, Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells, *J. Virol.* **67:**4945.

Wain-Hobson, S., 1992, Human immunodeficiency virus type 1 quasispecies *in vivo* and *ex vivo*, *Curr. Topics Microbiol. Immunol.* **176:**181.

Weichs an der Glon, C., Monks, J., and Proudfoot, N. J., 1991, Occlusion of the HIV poly(A) site, *Genes Dev.* **5:**244.

Weiss, R. A., 1993, Cellular receptors and viral glycoproteins involved in retrovirus entry, in: *The Retroviridae*, Vol. 2 (J. A. Levy, ed.), pp. 1–108, Plenum Press, New York.

Weiss, S., König, B., Morikawa, Y., and Jones, I., 1992, Recombinant HIV-1 nucleocapsid protein p15 produced as a fusion protein with glutathione S-transferase in *Escherichia coli* mediates dimerization and enhances reverse transcription of retroviral RNA, *Gene* **121:**203.

Whitcomb, J. M., and Hughes, S. M., 1992, Retroviral reverse transcription and integration: Progress and problems, *Annu. Rev. Cell Biol.* **8:**275.

Willbold, D., Rosin-Arbesfeld, R., Sticht, H., Frank, R., and Rosch, P., 1994, Structure of the equine infectious anemia virus Tat protein, *Science* **264:**1584.

Willey, R. L., Bonifacino, J. S., Potts, B. J., Martin, M. A., and Klausner, R. D., 1988, Biosynthesis, cleavage, and degradation of the human immunodeficiency virus 1 envelope glycoprotein gp160, *Proc. Natl. Acad. Sci. USA* **85:**9580.

Willey, R. L., Buckler-White, A., and Strebel, K., 1994, Sequences present in the cytoplasmic domain of CD4 are necessary and sufficient to confer sensitivity to the human immunodeficiency virus type 1 Vpu protein, *J. Virol.* **68:**1207.

Wills, N. M., and Craven, R. C., 1991, Form, function, and use of retroviral Gag proteins, *AIDS* **5:**639.

Wlodawer, A., Miller, M., Jaskolski, M., Sathyanarayana, B. K., Baldwin, E., Weber, I. T., Selk, L. M., Clawson, L., Schneider, J., and Kent, S. B. H., 1989, Conserved folding in retroviral proteases: Crystal structure of a synthetic HIV-1 protease, *Science* **245:**616.

Wong-Staal, F., Chanda, P. K., and Ghrayeb, J., 1987, Human immunodeficiency virus: The eighth gene, *AIDS Res. Hum. Retroviruses* **3:**33.

Xiong, Y., and Eickbush, T. H., 1990, Origin and evolution of retroelements based upon their reverse transcriptase sequences, *EMBO J.* **9:**3353.

Yang, W., Hendrickson, W. A., Crouch, R. J., and Satow, Y., 1990, Structure of ribonuclease H phased at 2 Å resolution by MAD analysis of the selenomethyl protein, *Science* **249:**1398.

Yeager, C. L., Ashnum, R., Williams, R. K., Cardellichio, C. B., Shapiro, L. H., Look, A. T., and Holmes, K. V., 1992, Human aminopeptidase is a receptor for human coronavirus 229E, *Nature* **357:**420.

Yu, G., and Felsted, R. L., 1992, Effect of myristoylation on p27 nef subcellular distribution and suppression of HIV-LTR transcription, *Virology* **1887:**46.

Yu, X. F., Ito, S., Essex, M., and Lee, T. H., 1988, A naturally immunogenic virion-associated protein specific for HIV-2 and SIV, *Nature* **335:**262.

Yu, X. F., Matsuda, M., Essex, M., and Lee, T. H., 1990, Open reading frame *vpr* of simian immunodeficiency virus encodes a virion-associated protein, *J. Virol.* **64:**5688.

Yu, X. F., Yuan, X., Matsuda, Z., Lee, T. H., and Essex, M., 1992, The matrix protein of human immunodeficiency virus type 1 is required for incorporation of viral envelope protein into mature virions, *J. Virol.* **66:**4966.

Zapp, M. L., and Green, M. R., 1989, Sequence-specific RNA binding by the HIV-1 Rev protein, *Nature* **342:**714.

Zhao, L. J., Mukherjee, S., and Narayan, O., 1994, Biochemical mechanism of HIV-I Vpr function. Specific interaction with a cellular protein, *J. Biol. Chem.* **269:**15577.