# gp41, A Multifunctional Protein Involved in HIV Entry and Pathogenesis

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## Introduction

The entry of HIV into a target cell represents the key initial step in the replication cycle of this virus. The entry process involves a coordinated series of molecular interactions between the two components of the virus glycoprotein complex (gp120(SU)/gp41(TM)) and the two components of the receptor complex (CD4 and a chemokine receptor). It results in the fusion of the viral membrane with that of the host cell and the introduction of the viral replicative machinery into the cytoplasm. It has been well established over the past several years that conserved domains of gp120 come together to form the binding site for amino-terminal residues of the CD4 molecule (Moore et al., 1993). For most HIV and SIV isolates, this SU-CD4 interaction defines the cell type to which the virus binds and ultimately enters (Sattentau and Moore, 1993). Recent data have shown, however, that for the process of membrane fusion to proceed, a second interaction, with a member of the chemokine receptor family of multiple membrane-spanning proteins, must occur. Natural isolates of HIV-1 which primarily infect macrophages and monocytes but lack the ability to infect T-cell lines utilize CCR5 , while T cell-line adapted and syncytium-inducing (SI) viruses primarily utilize CXCR4 (reviewed in Berger, 1997; Doms and Peiper, 1997; Moore, 1997). Binding of the receptor complex results in conformational changes in both gp120 and in the TM protein gp41. By analogy with the hemagglutinin of influenza virus, the TM protein is thought to be the engine that drives the membrane fusion process, and it is this multifaceted molecule that is the subject of this review.

## Biosynthesis of Env

The SU and TM components of the envelope glycoprotein complex are synthesized, as a single, co-translationally glycosylated, polyprotein precursor (gp160), on ribosomes associated with the endoplasmic reticulum (ER). Individual precursor molecules, shown schematically in Figure 1, initiate folding, presumably in the presence of ER-associated chaperonins, and assemble to form an oligomeric structure (Hunter and Swanstrom, 1990). Whether this is a trimeric or tetrameric molecule remains unresolved, although recent data suggest that a trimer is the biologically relevant form of the oligomer and this would be consistent with the findings for other retroviruses (see below). Mutagenic studies in both HIV and Rous sarcoma virus suggest that it is regions within the TM domain that direct and stabilize the oligomerization process (Earl and Moss, 1993; Einfeld and Hunter, 1994; Einfeld and Hunter, 1997). It is likely that this region is structurally conserved between HIV and SIV, since mixed Env oligomers have been described (Doms et al., 1990). Surprisingly, the membrane-spanning domain is not required for oligomers to form, although it and regions within the cytoplasmic domain may provide stability to the structure (Earl and Moss, 1993; Einfeld and Hunter, 1988). Formation of an Env oligomer appears to be required for efficient transport out of the ER.

Shortly after the stable oligomer is formed, it is transported from the ER to the Golgi complex where the bulk of the mannose-rich oligosaccharide side chains are processed to hybrid/terminally glycosylated forms. In a late Golgi compartment, most likely the trans-Golgi network, the precursor is cleaved to gp120 and gp41 by a cellular proteinase. Furin and PC7 appear to be the most likely candidates for this action (Decroly et al., 1997; Hallenberger et al., 1997). These enzymes, members of the subtilysin family of proteinases, recognize and cleave at a highly conserved basic sequence (RXXR) that links the C-terminus of gp120 to the N-terminus of gp41. Cleavage is important since it frees the fusion peptide at the amino-terminus of gp41 from the C-terminus of gp120, which is critical for its

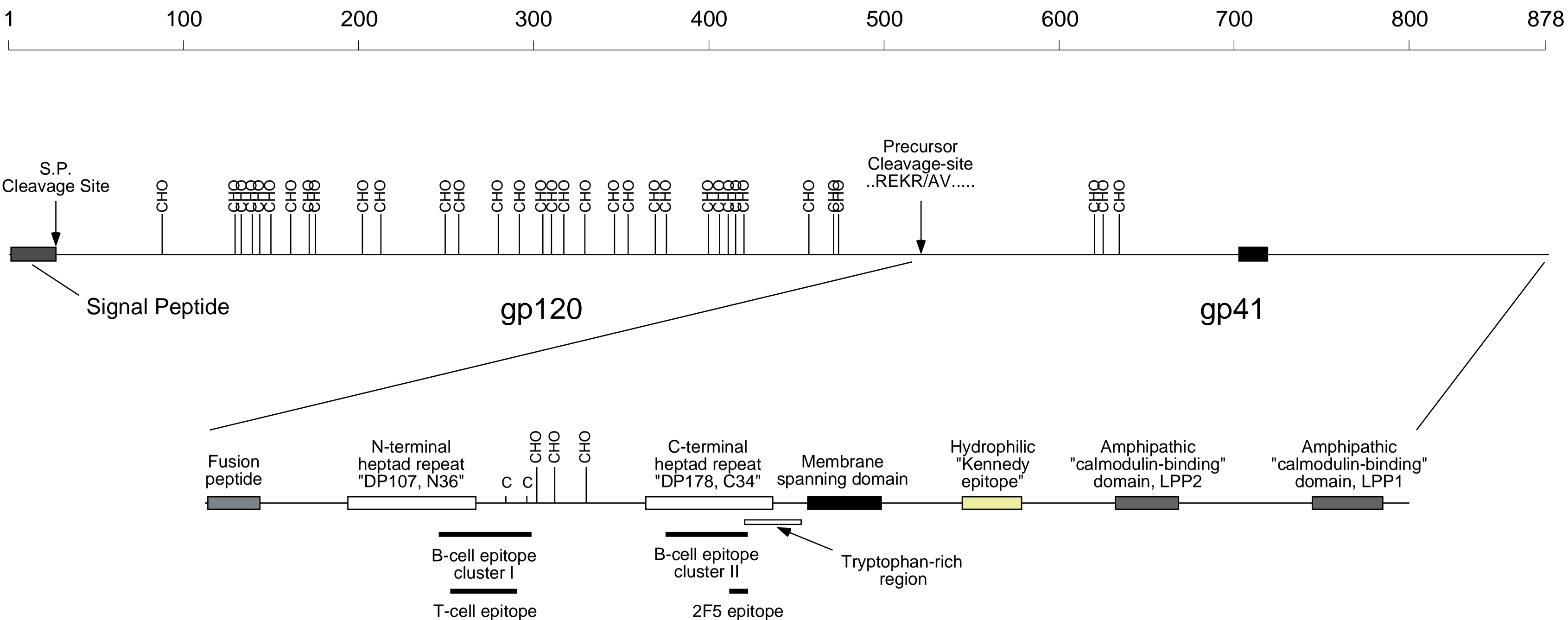


Figure 1. Organization of the HIV-1 env gene product and functional domains of gp41. The env open reading frame is depicted with vertical arrows showing sites for cleavage by the host-encoded signal peptidase and furin-like Golgi proteinase. Oligosaccharide addition sites that are utilized *in vivo* are shown by vertical lines and CHO. The position and size of critical functional domains as well as T- and B-cell epitopes are shown to scale. The position of the cysteine-bounded loop that is part of epitope Cluster I is shown as two upper case Cs.

future role in fusion. It is also probably accompanied by conformational changes in the oligomeric complex since uncleaved gp160 is inefficiently incorporated into virions (Bosch and Pawlita, 1990; Dubay et al., 1995). A post-cleavage conformational change is consistent with studies on influenza virus, where, in the crystal structure of the cleaved hemagglutinin, the C-terminus of HA1(SU) and the N-terminus of HA2(TM) are separated by 21 Å (Wilson et al., 1981). In the case of HIV Env, gp120 and gp41 are non-covalently associated following cleavage of the precursor. It can be concluded from an analysis of mutant glycoproteins that residues within conserved regions (C1 and C5) at the N-terminus and at the C-terminus of gp120 play a key role in this association (Helseth et al., 1991). In gp41, a region (residues 528–562) immediately carboxy-terminal to the fusion peptide is important for this association, although mutations outside this region can also be disruptive (Cao et al., 1993).

It is important to emphasize that the stable oligomeric structure, which forms in the ER, is folded from uncleaved polyprotein precursors. This clearly allows the folding of regions of both SU and TM into domains that are structurally constrained in a manner that would be impossible if they were assembled from the mature proteins, gp120 and gp41 (see below). If one considers the folding of this constrained structure as the setting of a spring-loaded trap, cleavage of the precursor might then be analogous to the removal of the safety catch.

## Immune Responses to gp41

Although the bulk of gp41 might be expected to be sequestered from the humoral immune system within the Env oligomeric complex, several regions of this molecule are recognized by sera from individuals infected with HIV. The first of these regions, often referred to as Cluster I extends from around residue 588 to residue 615 of gp41. The immunodominant epitope is centered around a short disulfide-bonded loop (residues 607–613) that is located within the central region of the gp41 ectodomain (1987b; Gnann et al., 1987c). Almost all sera from infected individuals react with this epitope even though some sequence diversity has been found in viruses of the O-subtype (Eberle et al., 1997). Nevertheless, antibodies to this epitope are not neutralizing and do not block infection by HIV. On the contrary, monoclonal antibodies reactive with this region have been reported to enhance infection through a complement-dependent mechanism (Robinson et al., 1990a; Robinson et al., 1990b). The immunologically active form of the seven-residue peptide, CSGKLIC, contains an intramolecular disulfide bond and NMR studies showed that this domain maintains a preference for a folded conformation, including a type I reverse turn about the residues SGKL. The presence of the disulfide bond is integral to the formation of the structure of the loop in solution. Mutations (Cys to Ser) which eliminate the possibility of loop formation result in the failure of antibody binding to this peptide (Oldstone et al., 1991).

In addition to this primary immunodominant epitope, a second ectodomain region, located proximal to the membrane-spanning region of gp41, is also recognized by sera from infected individuals (Cluster II; Horal et al., 1991; Xu et al., 1991). While a majority of antibodies reactive with this region do not neutralize HIV infection, one human monoclonal antibody (2F5) has been identified that neutralizes a variety of laboratory strains and clinical isolates of HIV-1. The amino acid sequence ELDKWA (residues 671–676) has been defined as the epitope recognized by this antibody (Muster et al., 1993). Sequence analysis of a variety of primary isolates suggests that the major determinant of MAb 2F5 binding corresponds to the amino-acid sequence LDKW, since naturally occurring and in vitro selected neutralization-resistant viruses contain changes in the D and K positions of the ELDKWA motif (Purtscher et al., 1996). While 2F5 recognizes a putatively linear determinant present on short peptides, it seems likely that the epitope reflects a specific conformation of the contributing amino acids, since attempts to elicit neutralizing antibodies to a synthetic peptide encompassing this region or to the epitope presented on the hepatitis B surface antigen have been unsuccessful (Eckhart et al., 1996). However, insertion of this epitope into the loop of antigenic site B of the influenza virus hemagglutinin did elicit ELDKWA-specific, neutralizing immunoglobulins in antisera of mice (Muster et al., 1994). The mechanism of neutralization is not understood, although Neurath and colleagues have reported multiple effects of binding the 2F5 monoclonal antibody to virions that are suggestive of post-binding conformational changes in Env (Neurath et al., 1995). It is also relevant that the 2F5 epitope overlaps the amino acid sequence of a peptide derived from gp41 that is a potent inhibitor of fusion (see below)

and a tryptophan-rich region that we have recently shown plays a critical role in fusion and infectivity (Salzwedel and Hunter, unpublished).

An enigma, as far as anti-gp41 neutralizing antibodies are concerned, has been the fact that monoclonal antibodies raised against a hydrophilic peptide corresponding to residues 743–760 (DR-PEGIEEEGGERDRDRS) of the cytoplasmic domain could neutralize a variety of HIV-1 isolates. Both monoclonal antibodies and polyclonal antisera raised against this epitope have neutralizing potential (Dalgleish et al., 1988; Evans et al., 1989). There is currently no evidence that this region of the cytoplasmic domain can be translocated to the outer surface of the cell and so how such antibodies can neutralize has remained a puzzle. Recent experiments, however, indicate that a monoclonal antibody to this epitope can bind with equal efficiency to infected and uninfected cells (Sattentau et al., 1995), suggesting that the epitope is also present on a cell surface protein. Since it is clear that the lipid envelope of HIV incorporates a large number of cell surface proteins (Arthur et al., 1992), it is possible that neutralization by antibodies to this epitope is mediated through one of these cell-derived molecules.

The TM protein also elicits activity from the cellular arm of the immune response. One of the CTL epitopes, identified through the use of synthetic peptides and fusion proteins carrying portions of the HIV-1 env gene, overlaps with the immunodominant B-cell epitope (amino acids 591–602 of the most-likely HIV env sequence - AVERYLKDQQLL). Only two positions within this epitope showed variation among North American HIV-1 isolates, and the substitutions were conservative in nature (Johnson et al., 1992). Interestingly, a Lys to Arg substitution at position 597 abolished recognition, probably by interfering with the peptide-MHC interactions (Dai et al., 1992; Hammond et al., 1991). In additional studies, CTL from four subjects recognized at least three distinct epitopes in this region of gp41 in the context of the MHC class 1 alleles A24, B8, B14, and B27 (Shankar et al., 1996). The other region of gp41 recognized by CTL is a stretch of 20 amino acids at the C-terminus of the cytoplasmic domain. CTL from seven subjects recognized epitopes within this region that were restricted by A30, B7, B8 and B35 class 1 alleles (Shankar et al., 1996).

## Role of gp41 in membrane fusion

### a) Conformational changes on receptor binding

Several enveloped viruses including influenza virus, vesicular stomatitis virus, and the alpha-viruses require the acidic environment of the endosome to activate the fusogenic potential of their envelope glycoprotein complexes. Thus after binding a receptor molecule on the cell surface, they are taken up into coated pits, undergo endocytosis and then release their replicative machinery into the cell following fusion with the endosomal membrane. In contrast the entry of HIV is not dependent on exposure to an acidic environment (McClure et al., 1988; Sinangil et al., 1988; Stein et al., 1987).

It is known, for the hemagglutinin of influenza virus and the glycoprotein complex of Semliki Forest virus, that the acid environment of the endosomal compartment induces a conformational change in the proteins that is necessary for activation of the fusion process (reviewed in White et al., 1983; White, 1992). In the case of HIV, and SIV analogous conformational changes have been observed following binding of receptor molecules (Sattentau and Moore, 1991; Sattentau et al., 1993), and recent experiments suggest that these receptor-induced conformation changes may parallel in molecular terms those induced by acid pH in the influenza virus HA.

Initially, experiments aimed at defining the mechanism by which a soluble form of the HIV receptor (sCD4) neutralized the infectivity of HIV demonstrated that treatment of laboratory strains of this virus with sCD4 could induce rapid shedding of gp120 and loss of the characteristic surface spikes seen in EMs of virions (Kirsh et al., 1990; Moore et al., 1990). Dissociation was temperature dependent and no significant dissociation of gp120 was observed between 4° C and 16° C (Hart et al., 1991; Moore and Klasse, 1992; Moore et al., 1991; Sattentau and Moore, 1991).

In the case of influenza virus HA, brief treatment of the virus with low pH, which activates fusion, results in the exposure of antigenic epitopes and protease cleavage sites that are normally sequestered within the HA molecule (Daniels et al., 1983; Skehel et al., 1982; White et al., 1983). Similarly,

dissociation of gp120 by CD4 is accompanied by exposure of epitopes on gp41 that are not reactive in the native molecule (Hart et al., 1991; Sattentau and Moore, 1991). Epitope exposure is not dependent upon loss of gp120 since similar results were obtained following CD4 binding at 4° C (Sattentau and Moore, 1991; Sattentau et al., 1995). A majority of studies have employed sCD4 treatment of HIV virions or infected cells, but Sattentau and Moore (1993) have demonstrated a similar exposure of gp41 epitopes at the interface of fusing cells. Thus membrane anchored CD4, like sCD4, can induce gp41 epitope exposure following gp120 binding.

The exposure of epitopes in both TM and SU, coupled with the dissociation of gp120 that has been observed with laboratory adapted isolates of HIV-1, appears to represent the most dramatic effect of sCD4 on Env conformation and may reflect the relative instability of the glycoprotein complexes of these isolates. The susceptibility of primary isolates of HIV-1 to sCD4 neutralization is significantly lower than that of cell-line adapted isolates (Daar et al., 1990) and sCD4 is very inefficient at inducing gp120 dissociation in the non-adapted strains (Moore et al., 1992), perhaps reflecting the lower affinity for CD4 exhibited by the oligomeric form of Env from natural isolates (Moore et al., 1992).

The glycoproteins of HIV-2 and SIV also have a lower affinity for CD4 and these viruses are similarly resistant to neutralization by sCD4. In contrast to the laboratory adapted strains of HIV-1, both SIV and HIV-2 infection and syncytium formation can be enhanced by sub-inhibitory concentrations of the soluble receptor. Incubation of sCD4 with SIVagm viruses not only accelerates the kinetics of infection but also leads to a 100-fold increase in the titer of the virus (Allan et al., 1990; Clapham et al., 1991; Sekigawa et al., 1990; Werner et al., 1990). A similar enhancement of infectivity has recently been observed with HIV-1 when poorly neutralizable primary isolates are treated with sub-inhibitory concentrations of sCD4 (Sullivan et al., 1995). Treatment of SIV or HIV-2 infected cells with the soluble receptor does not result in dissociation of gp120 or increased exposure of TM epitopes - apparently as a consequence of stronger interactions between the subunits in these viruses (Allan et al., 1992; Sattentau et al., 1993). Nevertheless, it does result in some conformational changes, since an increase was observed in the exposure of the V2 and V3 loops, as detected by antibody binding (Sattentau et al., 1993), and antibodies from SIVagm infected monkeys, which failed to neutralize virus in vitro, could neutralize the sCD4-induced enhancement of SIVagm infection (Allan et al., 1990). This is consistent with the suggestion that sCD4 enhancement of SIVagm (and presumably of HIV-1 primary isolates and HIV-2) is a form of receptor mediated activation (Allan et al., 1990), analogous to the low-pH activation observed with influenza virus or Semliki Forest virus (Marsh and Helenius, 1989).

**b) Molecular rearrangements following receptor binding - formation of coiled-coils**

Both crystallographic and peptide studies with influenza virus have shown that the low pH-mediated conformational changes that occur in HA involve the structural transition of a region within the HA2 (TM) protein. Thus residues C-terminal to the fusion peptide, that are constrained as an extended polypeptide chain within the inactive HA, form an elongated helical structure following low pH activation (Bullough et al., 1994; Carr and Kim, 1993). The newly formed helices of the HA trimer form a triple-helix, coiled-coil structure that is postulated to position the fusion peptide, located at the N-terminus of HA2, for insertion into the target cell membrane. A heptad repeat (leucine-zipper-like) region capable of forming a similar coiled-coil structure is found within the N-terminal 100 amino acids of the TM protein of a majority of retroviruses. Peptides corresponding to this domain form stable coiled-coil structures (Wild et al., 1992) and fusion of the heptad repeat to the C-terminus of a monomeric form of bacterial protein-A or maltose-binding protein results in assembly of oligomeric chimeric proteins (Bernstein et al., 1995; Shugars et al., 1996). Thus this region has the capability to both oligomerize and mediate oligomerization of protein molecules. The heptad repeat domain is highly conserved among HIV-1 isolates and was originally postulated to play a role in the assembly oligomerization of the glycoprotein complex that occurs in the ER (Delwart and Mosialos, 1990; Gallaher et al., 1989). Mutational analyses of the region have argued against a direct role in oligomer assembly in the ER but showed that even minor amino acid substitutions had dramatic effects on the biological activity of the HIV-1 glycoprotein, by blocking cell-cell fusion and virus infectivity (Chen, 1994; Chen et al., 1993; Dubay et al., 1992). Peptides corresponding to this region are potent inhibitors of HIV-1 induced cell fusion as well as virus infection, and this property correlates directly with the ability of the peptides to

form stable coiled-coil structures; the same mutations which block the biological function of Env also reduce the melting temperature of the peptide's coiled-coil structure as well as abrogate their inhibitory potential (Wild et al., 1994b). We have postulated therefore that binding of CD4 to gp120 might induce equivalent conformational changes to that seen during low pH treatment of the influenza virus HA, with the resulting formation of an extended coiled-coil that can present the N-terminal fusion peptide to the target cell membrane (Figure 2A and 2B) (Bernstein et al., 1995; Wild et al., 1994b). Such a mechanism would explain why mutations which prevent cleavage of the Env precursor effectively block fusion, since in the absence of a free amino-terminus, the fusion peptide would be unable to undergo such a dramatic displacement.

**c) Insertion of the fusion peptide**

While the formation of a coiled-coil structure might present the fusion peptide to the target cell membrane, the mechanism of action of the fusion peptide itself remains obscure. How, for example, does the amino-terminal fusion peptide of gp41 interact with the membrane and what molecular interactions must occur following insertion? Mutational analyses of the fusion peptides of HIV-1, HIV-2 and SIV, which show sequence homology to the fusion peptides of the paramyxoviruses (Gallaher, 1987), have demonstrated the importance of hydrophobic residues within this region; substitution of hydrophobic with hydrophilic residues reduces syncytium formation and virus infectivity, whereas enhancement of hydrophobicity increases both (Bosch et al., 1989; Freed and Myers, 1992; Freed et al., 1990; Steffy et al., 1992). Recent NMR analyses of the gp41 fusion domain indicated that a peptide corresponding to this region inserts into lipid micelles primarily as a helix (59%), with substantial beta-structure (26.7%). Fluorescence experiments with a tryptophan analogue of the peptide pointed to deep penetration of the peptide into the apolar hydrocarbon core. The region C-terminal to an alanine-15/glycine-16 flexible hinge interacts in a substantial way with the micelle, suggesting that it lies on the surface of the bilayer (Chang et al., 1997). Despite this data, it is still not clear if, in order to function during virus entry, the fusion peptide interacts only with lipid or whether it also interacts with membrane-associated proteins in a manner analogous to the signal peptides of secreted and membrane-spanning proteins.

**d) Structural analyses of gp41: implications for the fusion process**

Although the three-dimensional crystal structure of the influenza virus HA was established over 15 years ago (Wilson et al., 1981), similar attempts to determine the structure of the intact Env complex of HIV have been unsuccessful. Using the known structure of HA2 and structure-prediction algorithms, Gallaher (1989) proposed a secondary structure for the HIV TM that included a number of structural motifs common to the TMs of diverse retroviral isolates. While this model has been conceptually valuable, protein-structure prediction remains unreliable, particularly for membrane proteins, and, more importantly, it fails to take into account the potential for formation of intermediate, quasi-stable structures such as those observed in HA. Within the past two years, however, significant progress has been made in obtaining high-resolution structure information on the fusion-active form of the HIV TM.

Following expression of the ectodomain of gp41 in bacteria, the insoluble product could be converted to a soluble form through limited proteolysis. This generated two peptide fragments N51 and C43, derived from the N- and C-regions of the gp41 ectodomain respectively (Lu et al., 1995). The N51 peptide corresponds to the 4–3 heptad repeat region (residues 549–599) discussed previously, while the C43 peptide is derived from a C-terminal potentially helical region (residues 633–675). Biophysical studies showed that these two peptides associate to form a highly thermostable, helical, trimeric complex of heterotrimers in which the N51 and C34 helices are arranged in an anti-parallel fashion (Figure 3A; Lu et al., 1995). An almost identical, antiparallel, coiled-coil bundle could be isolated using the same approach with the SIV TM ectodomain (Blacklow et al., 1995). This provided the initial indication that the N- and C-terminal regions of TM can fold back onto themselves to form a stable, predominantly helical, complex. While these helical bundles lacked the interconnecting 33 amino acid region containing the immunodominant epitope (residues 600–632), expression of the intact gp41 ectodomain (minus the fusion peptide) in insect cells yielded soluble oligomeric (trimer) proteins that appear in the electron microscope to form similar rod-like structures and which by circular dichroism have high alpha-helical content (~80%) (Weissenhorn et al., 1996). Thus the presence of the

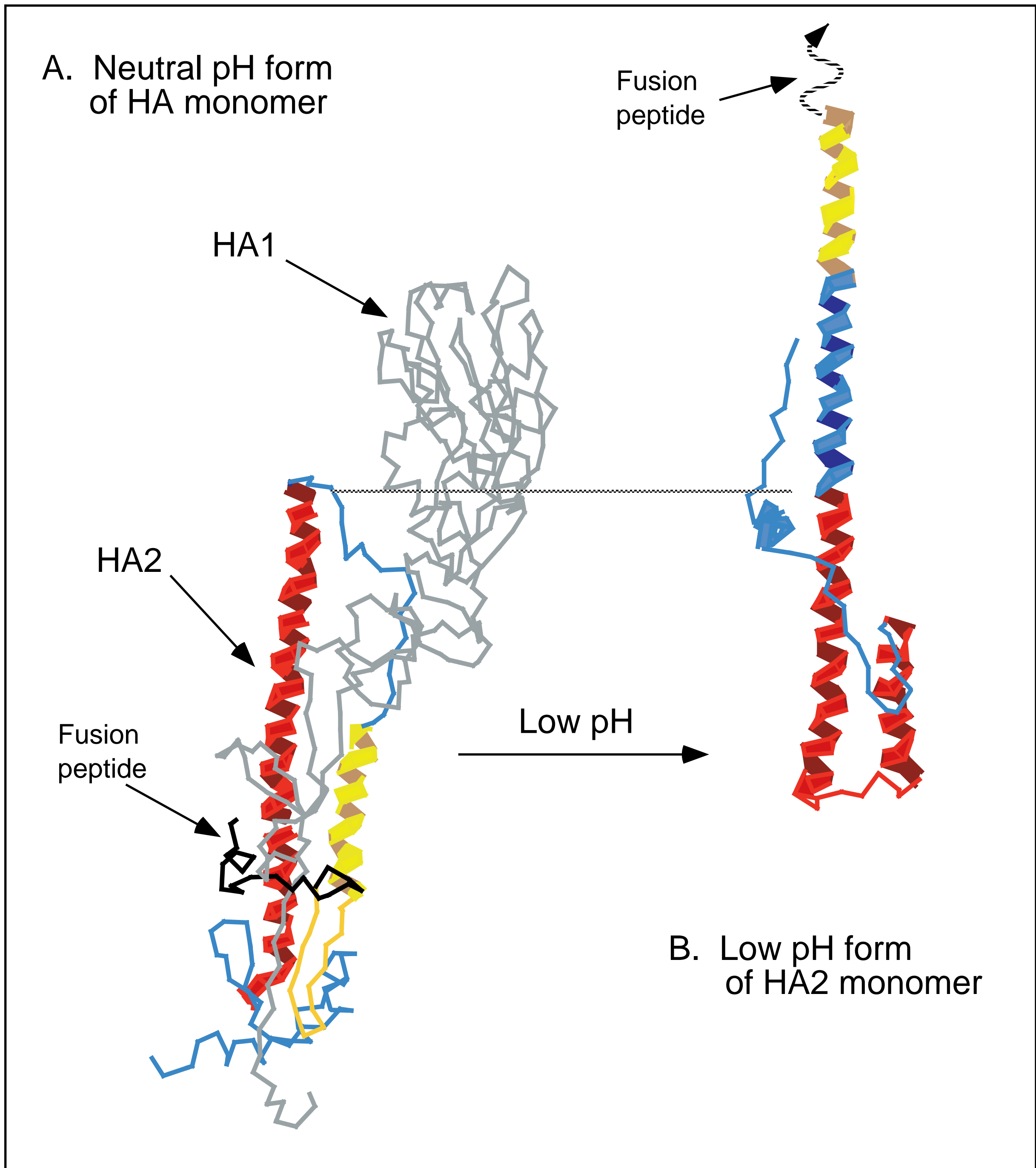


Figure 2. Ribbon diagram of the neutral and low pH forms of the influenza virus HA. **A**. Neutral pH form of the HA monomer crystal structure - HA1 is shown in grey, HA2 in colors that correspond to the amino terminal helix, the central extended chain and the carboxy-terminal helix (Wilson et al., 1981). **B**. Crystal structure of the low pH form of HA2 showing the formation of an extended helix following a major conformational change (Bullough et al., 1994). Note that the region of extended polypeptide chain between the N-terminal (yellow) helix and the C-terminal (red) helix is incorporated into the extended helix following acidification. The fusion peptide can thus be moved approximately 100Å.

Figure 3. Coiled-coil bundle formed by the N-terminal and C-terminal helical domains of gp41. Panel A shows proposed packing of helices in protease-resistant core from bacterially expressed gp41. The 33 amino-acid connecting loop with cysteine-bonded loop that is missing from the structure determined by Lu et al., (1996) is included schematically in this figure. Panel B shows a ribbon diagram of the coiled coil bundle formed by N36(555–590) and C34(637–670) peptides from gp41. Panel C shows a side-view of the space-filling model of the same structure (from the coordinates of Chan et al., 1997). White spheres are water molecules.

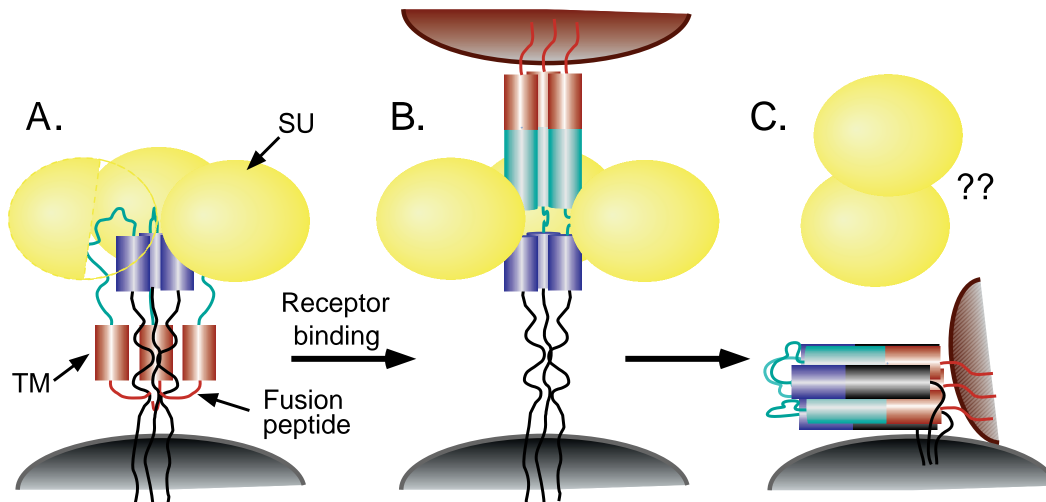


Figure 4. Hypothetical two-stage model for the conformational changes resulting in membrane proximity during fusion. **A**. Model for trimeric HIV Env complex. One SU molecule is cut away to show N-terminal heptad repeat constrained in an unstructured form. **B**. Following binding of the receptor complex, the extended-chain, heptad repeat region (shown in cyan) can assemble into a trimeric coiled-coil, thereby allowing the fusion peptide to insert into the target cell membrane. **C**. A second (rate limiting?) step in the conformational change is the formation of the coiled-coil bundle which results in apposition of the viral and target cell membranes. Peptide inhibitors might act by competing for the C-terminal heptad repeat domain during this latter step.

cysteine-loop region does not appear to interfere with the anti-parallel packing of the N- and C-terminal helices.

In the past year, two groups have succeeded in obtaining high resolution crystallographic data on the helical bundle formed by the N- and C-terminal domains of gp41. Chan et al. (1997) determined the structure of a slightly smaller complex, N36 (residues 555–590)/C34 (residues 637–670), to 2.0 Å resolution. This complex is a six-stranded helical bundle (fig 3B). The center of the bundle consists of a trimeric coiled-coil of three N36 helices arranged in parallel in a left-handed super helix. Three C34 helices are wrapped, antiparallel to the N36 helices, in a left-handed direction around the central coiled-coil trimer (Fig 3C). Overall the complex comprises a cylinder that is 35 Å in diameter and 55 Å in height. Using a somewhat different approach, Weissenhorn et al. (1997) determined the structure of the protease-resistant part of the gp41 ectodomain solubilized with a trimeric GCN4 coiled-coil in place of the amino-terminal fusion peptide. This structure, in which gp41 residues 550–597 form the triple-stranded alpha-helical coiled-coil core and residues 637–674 form the alpha-helices packed in the reverse direction against the outside of the coiled coil, is essentially identical to that of Chan et al. (1997). The C-terminal helices interact with the central coiled-coil core mainly through hydrophobic residues that are located in three grooves on its surface and the residues lining these grooves are highly conserved between HIV and SIV. It is important to note that peptides corresponding to the C-terminal helices do not have helical character in solution (Wild et al., 1995; Wild et al., 1994a) and so the formation and stability of these helices must depend to a large extent on their interaction with the triple-stranded central core. Indeed, it is quite possible that within the native Env complex on the virion, both the N-terminal heptad repeat region and the C-terminal domain are constrained as extended polypeptide chains, and that their transition to helical structure occurs only after receptor binding.

The trimeric coiled-coil complex has implications for the valency of the Env complex itself, for the structural rearrangements of Env involved in membrane fusion and for the development of novel antiretroviral drugs. The HIV Env complex has been described as a tetramer and as a dimer of dimers (Earl et al., 1990; Pinter et al., 1989; Schawaller et al., 1989) as well as a trimer (Gelderblom et al., 1987; Weiss et al., 1990). The trimeric nature of the gp41 coiled-coil complex, even though it is formed in the absence of gp120, clearly suggests that the native Env complex is also trimeric. This would be consistent with other retroviral Env complexes (Einfeld and Hunter, 1988; Einfeld and Hunter, 1990; Kamps et al., 1991) and with the influenza virus HA (Wilson et al., 1981).

The remarkable structural similarity of the HIV gp41 coiled-coil bundle to the fusion pH-induced conformation of influenza virus HA2 (Bullough et al., 1994) and to the transmembrane subunit of Moloney murine leukemia virus (Fass et al., 1997) led both Chan et al. (1997) and Weissenhorn et al. (1997) to propose that this structure represents the fusion-active form of the gp41 ectodomain. A number of factors support this conclusion. First the coiled-coil bundle is very thermostable, in contrast to the native Env complex, indicative of a terminal low energy conformation. Second, mutations that block fusion and infectivity map to residues that stabilize the coiled-coil bundle. For example the isoleucine at position 582, which we have shown is critical for fusion and for stability of the core triple helix (Wild et al., 1994b), makes a homotrimeric contact at the center of the coiled coil but also interacts with Trp640 in the outer helix . Similarly, Asn665 is in an a position (relative to the a-g positions of a heptad repeat) on the outer helix and packs into the groove on the central core. Mutation of this residue would be predicted to disrupt an extensive hydrogen bonding network that stabilizes the outer helix and, as might be expected if this were the case, abrogates fusion (Cao et al., 1993). Finally peptides corresponding to the N- and C-terminal helical domains that make up the coiled-coil bundle have potent anti-viral activity and block HIV fusion (Jiang et al., 1993; Lu et al., 1995; 1994b; Wild et al., 1993; 1994a). Given the stability of the coiled-coil structure, these peptides would not be expected to exert their antiviral effect once it has formed. In contrast, the structure supports the concept that the inhibitory peptides function by interacting with gp41 during the conformational changes to the fusion-active state. Thus the N-terminal peptides might block the formation of the internal coiled-coil or interact (as oligomeric peptides) with the C-terminal region, preventing its interaction with the N-terminal domain. C-terminal peptides might act competitively to prevent binding of this region of gp41 into the grooves of the central core. Indeed, a peptide comprising residues 647–682 (DP178) can bind to a bacterially produced gp41 ectodomain that has been deleted in this region. Moreover, the formation of this "molecular clasp"

reconstituted an epitope recognized by a monoclonal antibody prepared against the intact ectodomain of gp41 (Chen et al., 1995).

Taken together with the high resolution structural information, the data from mutations and inhibitory peptides points to a model for Env-induced fusion which involves two interdependent conformational changes in gp41 (Fig 4). In the first stage, following binding of the receptor complex, the constrained N-terminal heptad-repeat region of gp41 would be freed to assemble into the central coiled-coil, moving the fusion peptide in the process to a position where it could interact with the target cell membrane. The second stage would then be the formation of the coiled-coil bundle. Since this latter structure is arranged such that the N-terminal fusion peptide and the C-terminal membrane-spanning domain of gp41 are at the same end of the bundle, its formation would force the target cell membrane and the viral membrane in close proximity. This mechanical process thus might facilitate the initiation of the process where lipid mixing occurs. Neither the source of energy for the second conformational change and membrane apposition, which would be expected to have a high DG, nor the time frame over which such a process might occur is obvious. However, the fact that peptides such as DP178 can inhibit the fusion process at very low concentrations (pM), suggests that the formation of the coiled-coil bundle might be a rate limiting step in fusion.

**e) gp41/fusion as an anti-viral target**

It is clear from the work of several groups that peptides corresponding to the N-terminal heptad repeat (DP107) and the C-terminal helical domain (DP178, C34) represent potent inhibitors of HIV-1 entry and replication (Jiang et al., 1993; Lu et al., 1995; 1994b; Wild et al., 1993; 1994a). The possible mechanism of action of these peptides is discussed above and suggests that the conformational changes in gp41 are a novel target for anti-HIV therapeutics. A particularly important development of this concept, therefore, has been the initial clinical trial of the C-terminal (DP178) peptide in HIV-1 infected patients. In this study, 5 of 5 patients, receiving 100mg of the peptide twice daily for a period of 17 days, showed greater than 100-fold reductions in viral load (Saag et al., 1997) - an anti-viral effect greater than several RT inhibitors. Thus it appears that the fusion/entry process is a viable in vivo therapeutic target. The presence of the highly conserved, deep cavities on the N-terminal coiled-coil trimer with which conserved C-terminal residues interact, raises the possibility that novel small molecule or peptidomimetic inhibitors of HIV infection might be developed (Chan et al., 1997; Weissenhorn et al., 1997).

**f) Requirement for a membrane anchor**

The membrane-spanning anchor region of the TM protein is an important structural domain, as might be expected if the initial result of fusion peptide insertion is an oligomeric structure that acts as a bridge between the viral and cell membranes. The region of gp41 that spans the membrane to yield a fusion-active form of the HIV-1 Env remains ill-defined, but C-terminal truncations indicate residues 692–716 can function as a membrane anchor (Dubay et al., 1992; Helseth et al., 1990; Johnston et al., 1993). Point mutations and small deletion mutations within this region can abrogate fusion without affecting the membrane-anchor function (Helseth et al., 1990; Owens et al., 1994). Nevertheless, because the membrane-spanning and cytoplasmic domains of the HIV-1 glycoprotein can be replaced by those of CD4 or CD28 without loss of fusogenicity (Vincent et al., 1993; Wilk et al., 1996), there appears to be no requirement for a specific amino acid sequence within the transmembrane domain for Env to mediate the fusion reaction. In contrast, substitution of a glycosylphosphatidylinositol (GPI) anchor for the HIV-1 protein anchor abrogates fusion and infectivity, even though this GPI-linked protein is efficiently transported to the plasma membrane and incorporated into virions (Salzwedel et al., 1993; Weiss and White, 1993). A similar result has been reported for a GPI-linked form of the influenza virus HA protein, although in this case lipid mixing of the outer leaflet of the bilayer (hemi-fusion) was seen in the absence of complete fusion and mixing of cell contents (Kemble et al., 1994). Thus a protein component that spans both leaflets of the lipid bilayer appears to be critical for complete mixing of the viral and target membranes. Weissenhorn et al. (1997) have suggested that the rod-shaped gp41 coiled-coil bundles might cluster at their hydrophobic tips (which includes the membrane-spanning domain), thereby forming asters with centers that could be the points of initial membrane fusion.

## Role of the HIV/SIV Cytoplasmic Domain

The TM proteins of lentiviruses have unusually long cytoplasmic domains. In HIV and SIV they exceed 150 amino acids and in the case of Visna virus it is more than 200 amino acids in length. This contrasts with most retroviral TMs where the cytoplasmic domain ranges from 20–40 amino acids (Hunter and Swanstrom, 1990). In an attempt to define the biological role of the additional residues in the cytoplasmic domain, several investigators have introduced premature termination codons in both the HIV-1 and SIV Env coding sequence to progressively truncate the cytoplasmic domain. The results of these experiments indicate that the cytoplasmic domain is dispensable for HIV or SIV Env-mediated cell-cell fusion. In the case of HIV-1, however, truncation of the cytoplasmic domain by as few as 20 amino acids results in significantly decreased virus replication in most cell types, although the virus is still viable in the highly permissive MT4 cell line (Dubay et al., 1992; Freed and Martin, 1995; Gabuzda et al., 1992; Wilk et al., 1992). SIV replication, in contrast, appears to have no requirement for the cytoplasmic domain of Env (Johnston et al., 1993; Zingler and Littman, 1993). Indeed growth of SIV in human cells results in the selection for a viral Env with a spontaneous truncation which broadens the host range of the virus, probably by increasing the number of Env molecules incorporated into virions (Johnston et al., 1993) (Zingler and Littman, 1993). Nevertheless, reversion back to wild-type virus has been observed following inoculation of virus encoding the Env truncation into macaques, suggesting that in vivo this region plays an important role (P. Luciw, personal communication; Kodama et al., 1989). A number of structural elements within the cytoplasmic domain may contribute to this in vivo function.

## Endocytosis/Basolateral Targeting Signals

Cytoplasmic amino-acid motifs can function to direct endocytosis of a protein from the plasma membrane (reviewed in Trowbridge et al., 1993). Over the past few years, the sequences of these internalization/targeting signals have been identified for several constitutively reycling cell surface receptors and have been shown to involve tyrosine residues within a tight turn of the peptide backbone (Robinson et al., 1996; Trowbridge et al., 1993). For the transferrin receptor (TR) this sequence is Tyr-X-Arg-Phe and a general consensus for this type of internalization motif is Tyr -X-Arg/Lys-Y, where Y is usually a hydrophobic amino acid (Collawn et al., 1990). We noted previously (Hunter, 1994) that a majority of retroviral cytoplasmic domains contained a similar motif. In HIV and SIV, a Tyr-X-Pro-Leu/Val sequence, located membrane-proximally (residues 721–724) within the cytoplasmic domain, is highly conserved. Hoxie and colleagues have described a spontaneous mutant of an SIV Env protein in which the tyrosine in this motif was substituted by a cysteine. This mutant was poorly endocytosed, and was expressed on the cell surface and incorporated into virions at high levels (LaBranche et al., 1995; Sauter et al., 1996). Similarly, Siliciano and colleagues have shown that this tyrosine containing motif is important for efficient endocytosis of the HIV-1 Env molecule (Rowell et al., 1995). Additionally, Sauter et al. (1996) were able to demonstrate directly the role of the SIV motif in endocytosis by substituting the cytoplasmic domain of Env for that of CD4.

What is the rationale for conserving signals that result in the internalization and degradation of a protein that must be incorporated into virions for infectivity? It has been proposed that this process might reduce immunological recognition of infected cells; alternatively, the motifs might play a role in correct intracellular targeting. In epithelial cells, where the apical and basolateral membranes have distinct protein populations, retroviral glycoproteins are specifically targeted to the basolateral surface (Roth et al., 1983). When HIV-1 Gag and Env are co-expressed in epithelial cells, it is at this surface that virus assembly and release occurs (Lodge et al., 1994; Owens et al., 1991). Mutation of the same tyrosine-containing motif that directs endocytosis abrogates this basolateral targeting of HIV-1 Env and basolateral assembly of virions (Lodge et al., 1997).

Interestingly, the rate of internalization of Env protein from the surfaces of HIV-1-infected cells is slow, and the presence of the Pr55gag precursor protein appears to be necessary and sufficient to inhibit Env internalization (Egan et al., 1996). Thus an interaction of the matrix domain of the Gag protein with Env during assembly may preclude the interaction of Env with host adaptin molecules that recruit plasma membrane molecules into clathrin-coated pits and thereby effect incorporation into virions.

### Calmodulin-binding domains

The cytoplasmic domains of HIV-1, HIV-2 and SIV contain two regions that would be predicted to fold into amphipathic helical segments which closely resemble those found in calmodulin-activated enzymes. Synthetic peptides corresponding to these regions have been shown to bind to calmodulin (CaM) with high affinity in the presence of calcium (Miller et al., 1993; Srinivas et al., 1993). These amphipathic peptides inhibit CaM-regulated activation of bovine brain phosphodiesterase in vitro, and have also been shown to inhibit mitogen-induced lymphocyte activation, a property shared by CaM antagonists. These properties are not an artifact of peptides since purified HIV-1 gp160 as well as full-length gp41 binds to CaM and expression of HIV Env in cells results in CaM co-localization (Radding et al., 1996). Moreover, while CaM binding by the C-terminal amphipathic helix is very sensitive to amino acid changes, the natural variation found in this region of Env leaves CaM-binding intact (Tencza et al., 1997; Tencza et al., 1995). Since calmodulin is intimately involved in the metabolism and functioning of T-cells, these studies point to a novel mechanism of viral cytopathogenesis mediated by the interaction of the HIV TM protein with cellular CaM, that could in turn uncouple critical cellular signal transduction pathways (Miller et al., 1993; Srinivas et al., 1993).

### Conclusions

Despite the wealth of new information summarized above, much remains to be learned about the role of gp41 in HIV/SIV replication and pathogenesis. The similarities between the HIV Env complex and the HA oligomer of influenza virus has allowed functional extrapolations to be drawn from the limited structural information obtained from bacterially expressed proteins. Nevertheless, even moderate resolution structural data on the native Env complex would provide information critical to understanding the extent of the conformational changes that have only been inferred to-date. High resolution three-dimensional structures of the native molecule would clearly assist in the development of small molecule inhibitors that could block the postulated conformational changes in gp41 following receptor binding and would thus interfere with the process of membrane fusion.

It seems likely that the long cytoplasmic domain of the lentiviruses plays a key role in viral pathogenesis. In SIV the cytoplasmic domain can be truncated without affecting virus replication in vitro, yet the conservation of endocytosis signals, CaM binding domains, as well as structural elements that interact with the assembling capsid precursors, suggests that this region might play multiple roles in both virus replication and pathogenesis in vivo. Experiments aimed at defining the structure of the cytoplasmic domain and the cell-encoded molecules with which it likely interacts could yield important information in this regard.

The HIV/SIV TM protein provides an excellent example of how multiple, complex functions can be packaged in a small molecule. Although much has been learned in the last few years about the function of gp41, even more remains to be uncovered. The next few years promise to yield exciting new information both on it and the molecular events involved in virus-cell membrane fusion.

### References

Allan, J. S., Strauss, J., and Buck, D. W. (1990). Enhancement of SIV infection with soluble receptor molecules. *Science* **247**:1084–8.

Allan, J. S., Whitehead, E. M., Strout, K., Short, M., Kanda, P., Hart, T. K., and Bugelski, P. J. (1992). Strong association of simian immunodeficiency virus (SIVagm) envelope glycoprotein heterodimers: possible role in receptor-mediated activation. *AIDS Res. Hum. Retroviruses* **8**:2011–20.

Arthur, L., Bess, J., Jr, Sowder, R., II, Benveniste, R., Mann, D., Chermann, J., and Henderson, L. (1992). Cellular proteins bound to immunodeficiency viruses: Implications for pathogenesis and vaccines. *Science* **258**:1935–1938.

Berger, E. A. (1997). HIV entry and tropism: the chemokine receptor connection. *AIDS* **11** (suppl A), S3–S16.

Bernstein, H. B., Tucker, S. P., Kar, S. R., Mcpherson, S. A., Mcpherson, D. T., Dubay, J. W., Lebowitz, J., Compans, R. W., and Hunter, E. (1995). Oligomerization of the hydrophobic heptad repeat of gp41. *J. Virol.* **69**:2745–2750.

Blacklow, S. C., Lu, M., and Kim, P. S. (1995). A trimeric subdomain of the simian immunodeficiency virus envelope glycoprotein. *Biochemistry* **34**:14955–62.

Bosch, M. L., Earl, P. L., Fargnoli, K., Picciafuoco, S., Giombini, F., Wong-Staal, F., and Franchini, G. (1989). Identification of the fusion peptide of primate immunodeficiency viruses. *Science* **244**:694–7.

Bosch, V., and Pawlita, M. (1990). Mutational analysis of the human immunodeficiency virus type 1 env gene product proteolytic cleavage site. *J. Virol.* **64**:2337–44.

Bullough, P. A., Hughson, F. M., Skehel, J. J., and Wiley, D. C. (1994). Structure of influenza haemagglutinin at the pH of membrane fusion. *Nature* (London) **371**:37–43.

Cao, J., Bergeron, L., Helseth, E., Thali, M., Repke, H., and Sodroski, J. (1993). Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein. *J. Virol.* **67**:2747–55.

Carr, C. M., and Kim, P. S. (1993). A spring-loaded mechanism for the conformational change of influenza hemagglutinin. *Cell* **73**:823–832.

Chan, D. C., Fass, D., Berger, J. M., and Kim, P. S. (1997). Core structure of gp41 from the HIV envelope glycoprotein. *Cell* **89**:263–73.

Chang, D. K., Cheng, S. F., and Chien, W. J. (1997). The amino-terminal fusion domain peptide of human immunodeficiency virus type 1 gp41 inserts into the sodium dodecyl sulfate micelle primarily as a helix with a conserved glycine at the micelle-water interface. *J. Virol.* **71**:6593–602.

Chen, C. H., Matthews, T. J., McDanal, C. B., Bolognesi, D. P., and Greenberg, M. L. (1995). A molecular clasp in the human immunodeficiency virus (HIV) type 1 TM protein determines the anti-HIV activity of gp41 derivatives: implication for viral fusion. *J. Virol.* **69**:3771–7.

Chen, S. S. (1994). Functional role of the zipper motif region of human immunodeficiency virus type 1 transmembrane protein gp41. *J. Virol.* **68**:2002–10.

Chen, S. S., Lee, C. N., Lee, W. R., McIntosh, K., and Lee, T. H. (1993). Mutational analysis of the leucine zipper-like motif of the human immunodeficiency virus type 1 envelope transmembrane glycoprotein. *J. Virol.* **67**:3615–9.

Clapham, P. R., Blanc, D., and Weiss, R. A. (1991). Specific cell surface requirements for the infection of CD4-positive cells by human immunodeficiency virus types 1 and 2 and by Simian immunodeficiency virus. *Virology* **181**:703–15.

Collawn, J. F., Stangel, M., Kuhn, L. A., Esekogwu, V., Jing, S. Q., Trowbridge, I. S., and Tainer, J. A. (1990). Transferrin receptor internalization sequence YXRF implicates a tight turn as the structural recognition motif for endocytosis. *Cell* **63**:1061–1072.

Daar, E. S., Li, X. L., Moudgil, T., and Ho, D. D. (1990). High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. *Proc. Natl. Acad. Sci. USA* **87**:6574–8.

Dai, L. C., West, K., Littaua, R., Takahashi, K., and Ennis, F. A. (1992). Mutation of human immunodeficiency virus type 1 at amino acid 585 on gp41 results in loss of killing by CD8+ A24-restricted cytotoxic T lymphocytes. *J. Virol.* **66**:3151–4.

Dalgleish, A. G., Chanh, T. C., Kennedy, R. C., Kanda, P., Clapham, P. R., and Weiss, R. A. (1988). Neutralization of diverse HIV-1 strains by monoclonal antibodies raised against a gp41 synthetic peptide. *Virology* **165**:209–15.

Daniels, R. S., Douglas, A. R., Skehel, J. J., and Wiley, D. C. (1983). Analyses of the antigenicity of influenza haemagglutinin at the pH optimum for virus-mediated membrane fusion. *J. Gen. Virol.* **64**:1657–1662.

Decroly, E., Benjannet, S., Savaria, D., and Seidah, N. G. (1997). Comparative functional role of PC7 and furin in the processing of the HIV envelope glycoprotein gp160. *Febs Lett* **405**:68–72.

Delwart, E. L., and Mosialos, G. (1990). Retroviral envelope glycoproteins contain a leucine zipper like repeat. *AIDS Res. Hum. Retroviruses* **6**:703–706.

Doms, R. W., Earl, P. L., Chakrabarti, S., and Moss, B. (1990). Human immunodeficiency virus types 1 and 2 and simian immunodeficiency virus env proteins possess a functionally conserved assembly domain. *J. Virol.* **64**:3537–40.

Doms, R. W., and Peiper, S. C. (1997). Unwelcomed guests with master keys: how HIV uses chemokine receptors for cellular entry. *Virology* **235**:179–90.

Dubay, J. W., Dubay, S. R., Shin, H. J., and Hunter, E. (1995). Analysis of the cleavage site of the human immunodeficiency virus type 1 glycoprotein: requirement of precursor cleavage for glycoprotein incorporation. *J. Virol.* **69**:4675–82.

Dubay, J. W., Roberts, S. J., Brody, B., and Hunter, E. (1992). Mutations in the leucine zipper of the human immunodeficiency virus type 1 transmembrane glycoprotein affect fusion and infectivity. *J. Virol.* **66**:4748–56.

Dubay, J. W., Roberts, S. J., Hahn, B. H., and Hunter, E. (1992). Truncation of the human immunodeficiency virus type 1 transmembrane glycoprotein cytoplasmic domain blocks virus infectivity. *J. Virol.* **66**:6616–25.

Earl, P. L., Doms, R. W., and Moss, B. (1990). Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein. *Proc. Natl. Acd. Sci. USA* **87**:648–652.

Earl, P. L., and Moss, B. (1993). Mutational analysis of the assembly domain of the HIV-1 envelope glycoprotein. *AIDS Res. Hum. Retroviruses* **9**:589–94.

Eberle, J., Loussert-Ajaka, I., Brust, S., Zekeng, L., Hauser, P. H., Kaptue, L., Knapp, S., Damond, F., Saragosti, S., Simon, F., and Gurtler, L. G. (1997). Diversity of the immunodominant epitope of gp41 of HIV-1 subtype O and its validity for antibody detection. *J. Virol. Methods* **67**:85–91.

Eckhart, L., Raffelsberger, W., Ferko, B., Klima, A., Purtscher, M., Katinger, H., and Ruker, F. (1996). Immunogenic presentation of a conserved gp41 epitope of human immunodeficiency virus type 1 on recombinant surface antigen of hepatitis B virus. *J. Gen. Virol.* **77**:2001–8.

Egan, M. A., Carruth, L. M., Rowell, J. F., Yu, X., and Siliciano, R. F. (1996). Human immunodeficiency virus type 1 envelope protein endocytosis mediated by a highly conserved intrinsic internalization signal in the cytoplasmic domain of gp41 is suppressed in the presence of the Pr55gag precursor protein. *J. Virol.* **70**:6547–56.

Einfeld, D., and Hunter, E. (1988). Oligomeric structure of a prototype retrovirus glycoprotein. *Proc. Natl. Acad. Sci. USA* **85**:8688–8692.

Einfeld, D., and Hunter, E. (1990). Oligomeric structure of retroviral envelope glycoproteins. In: *The use of X-ray crystallography in the design of antiviral agents*, W. G. Laver and G. M. Air, eds.: Academic Press), pp. 335–343.

Einfeld, D. A., and Hunter, E. (1994). Expression of the TM protein of Rous sarcoma virus in the absence of SU shows that this domain is capable of oligomerization and intracellular transport. *J. Virol.* **68**:2513–20.

Einfeld, D. A., and Hunter, E. (1997). Mutational analysis of the oligomer assembly domain in the transmembrane subunit of the Rous sarcoma virus glycoprotein. *J. Virol.* **71**:2383–2389.

Evans, D. J., McKeating, J., Meredith, J. M., Burke, K. L., Katrak, K., John, A., Ferguson, M., Minor, P. D., Weiss, R. A., and Almond, J. W. (1989). An engineered poliovirus chimaera elicits broadly reactive HIV-1 neutralizing antibodies. *Nature* **339**:385–388.

Fass, D., Harrison, S. C., and Kim, P. S. (1997). Retrovirus envelope domain at 1.7 angstrom resolution. *Nature* **388**:691–3.

Freed, E. O., and Martin, M. A. (1995). Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. *J. Virol.* **69**:1984–9.

Freed, E. O., and Myers, D. J. (1992). Identification and characterization of fusion and processing domains of the human immunodeficiency virus type 2 envelope glycoprotein. *J. Virol.* **66**:5472–8.

Freed, E. O., Myers, D. J., and Risser, R. (1990). Characterization of the fusion domain of the human immunodeficiency virus type 1 envelope glycoprotein gp41. *Proc. Natl. Acad. Sci. USA* **87**:4650–4.

Gabuzda, D. H., Lever, A., Terwilliger, E., and Sodroski, J. (1992). Effects of deletions in the cytoplasmic domain on biological functions of human immunodeficiency virus type 1 envelope glycoproteins. *J. Virol*. **66**:3306–15.

Gallaher, W. R. (1987). Detection of a fusion peptide sequence in the transmembrane protein of the human immunodeficiency virus. *Cell* **50**:327–328.

Gallaher, W. R., Ball, J. M., 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**:431–440.

Gelderblom, H. R., Hausmann, E. H., Ozel, M., Pauli, G., and Koch, M. A. (1987). Fine structure of human immunodeficiency virus (HIV) and immunolocalization of structural proteins. *Virology* **156**:171–6.

Gnann, J. W., Jr., Nelson, J. A., and Oldstone, M. B., gp41 epitopes, LA (1987b). Fine mapping of an immunodominant domain in the transmembrane glycoprotein of human immunodeficiency virus. *J. Virol.*. **61**:2639–41.

Gnann, J. W., Jr., Schwimmbeck, P. L., Nelson, J. A., Truax, A. B., and Oldstone, M. B., gp41 epitopes, LA (1987c). Diagnosis of AIDS by using a 12-amino acid peptide representing an immunodominant epitope of the human immunodeficiency virus. *J Infect Dis*. **156**:261–7.

Hallenberger, S., Moulard, M., Sordel, M., Klenk, H. D., and Garten, W. (1997). The role of eukaryotic subtilisin-like endoproteases for the activation of human immunodeficiency virus glycoproteins in natural host cells. *J. Virol*. **71**:1036–45.

Hammond, S. A., Obah, E., Stanhope, P., Monell, C. R., Strand, M., Robbins, F. M., Bias, W. B., Karr, R. W., Koenig, S., and Siliciano, R. F. (1991). Characterization of a conserved T cell epitope in HIV-1 gp41 recognized by vaccine-induced human cytolytic T cells. *J. Immunol*. **146**:1470–7.

Hart, T. K., Kirsh, R., Ellens, H., Sweet, R. W., Lambert, D. M., Petteway, S., Jr., Leary, J., and Bugelski, P. J. (1991). Binding of soluble CD4 proteins to human immunodeficiency virus type 1 and infected cells induces release of envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **88**:2189–93.

Helseth, E., Olshevsky, U., Furman, C., and Sodroski, J. (1991). Human immunodeficiency virus type 1 gp120 envelope glycoprotein regions important for association with the gp41 transmembrane glycoprotein. *J. Virol*. **65**:2119–23.

Helseth, E., Olshevsky, U., Gabuzda, D., Ardman, B., Haseltine, W., and Sodroski, J. (1990). Changes in the transmembrane region of the human immunodeficiency virus type 1 gp41 envelope glycoprotein affect membrane fusion. *J. Virol*. **64**:6314–8.

Horal, P., Svennerholm, B., Jeansson, S., Rymo, L., Hall, W. W., and Vahlne, A. (1991). Continuous epitopes of the human immunodeficiency virus type 1 (HIV-1) transmembrane glycoprotein and reactivity of human sera to synthetic peptides representing various HIV-1 isolates. *J. Virol*. **65**:2718–23.

Hunter, E. (1994). Macromolecular interactions in the assembly of HIV and other retroviruses. *Seminars in Virology* **5**:71–83.

Hunter, E., and Swanstrom, R. (1990). Retroviral envelope glycoproteins. In Retroviruses - Strategies for Replication, R. Swanstrom and P. K. Vogt, eds. (Berlin: Springer-Verlag), pp. 187–253.

Jiang, S., Lin, K., Strick, N., and Neurath, A. R. (1993). Inhibition of HIV-1 infection by a fusion domain binding peptide from the HIV-1 envelope glycoprotein GP41. *Biochem Biophys Res Commun* **195**:533–8.

Johnson, R. P., Trocha, A., Buchanan, T. M., and Walker, B. D. (1992). Identification of overlapping HLA class I-restricted cytotoxic T cell epitopes in a conserved region of the human immunodeficiency virus type 1 envelope glycoprotein: definition of minimum epitopes and analysis of the effects of sequence variation. *J. Exp. Med*. **175**:961–71.

Johnston, P. B., Dubay, J. W., and Hunter, E. (1993). Truncations of the simian immunodeficiency virus transmembrane protein confer expanded virus host range by removing a block to virus entry into cells. *J. Virol*. **67**:3077–86.

Kamps, C. A., Lin, Y. C., and Wong, P. K. Y. (1991). Oligomerization and transport of the envelope protein of Moloney murine leukemia virus-TB and of ts-1, a neurovirulent temperature-sensitive mutant of Mo-MuLV-TB. *Virology* **184**:687–694.

Kemble, G. W., Danieli, T., and White, J. M. (1994). Lipid-anchored influenza hemagglutinin promotes hemifusion, not complete fusion. *Cell* **76**:383–91.

Kirsh, R., Hart, T. K., Ellens, H., Miller, J., Petteway, S. J., Lambert, D. M., Leary, J., and Bugelski, P. J. (1990). Morphometric analysis of recombinant soluble CD4-mediated release of the envelope glycoprotein gp120 from HIV-1. *AIDS Res. Hum. Retroviruses* **6**:1209–12.

Kodama, T., Wooley, D. P., Naidu, Y. M., Kestler, H. W. d., Daniel, M. D., Li, Y., and Desrosiers, R. C. (1989). Significance of premature stop codons in env of simian immunodeficiency virus. *J. Virol.* **63**:4709–14.

LaBranche, C. C., Sauter, M. M., Haggarty, B. S., Vance, P. J., Romano, J., Hart, T. K., Bugelski, P. J., Marsh, M., and Hoxie, J. A. (1995). A single amino acid change in the cytoplasmic domain of the simian immunodeficiency virus transmembrane molecule increases envelope glycoprotein expression on infected cells. *J. Virol.* **69**:5217–27.

Lodge, R., Göttlinger, H., Gabuzda, D., Cohen, E. A., and Lemay, G. (1994). The intracytoplasmic domain of gp41 mediates polarized budding of human immunodeficiency virus type 1 in MDCK cells. *J. Virol.* **68**:4857–4861.

Lodge, R., Lalonde, J. P., Lemay, G., and Cohen, E. A. (1997). The membrane-proximal intracytoplasmic tyrosine residue of HIV-1 envelope glycoprotein is critical for basolateral targeting of viral budding in MDCK cells. *EMBO J.* **16**:695–705.

Lu, M., Blacklow, S. C., and Kim, P. S. (1995). A trimeric structural domain of the HIV-1 transmembrane glycoprotein. *Nat Struct Biol* **2**:1075–82.

Marsh, M., and Helenius, A. (1989). Virus entry into animal cells. *Adv Virus Res* **36**:107–51.

McClure, M. O., Marsh, M., and Weiss, R. A. (1988). Human immunodeficiency virus infection of CD4-bearing cells occurs by a pH-independent mechanism. EMBO J. **7**:513–8.

Miller, M. A., Mietzner, T. A., Cloyd, M. W., Robey, W. G., and Montelaro, R. C. (1993). Identification of a calmodulin-binding and inhibitory peptide domain in the HIV-1 transmembrane glycoprotein. *AIDS Res. Hum. Retroviruses* **9**:1057–66.

Moore, J. P. (1997). Coreceptors: implications for HIV pathogenesis and therapy. *Science* **276**:51–2.

Moore, J. P., Jameson, B. A., Sattentau, Q. J., Willey, R., and Sodroski, J. (1993). Towards a structure of the HIV-1 envelope glycoprotein gp120: an immunochemical approach. *Philos Trans R Soc Lond Biol* **342**:83–8.

Moore, J. P., and Klasse, P. J. (1992). Thermodynamic and kinetic analysis of sCD4 binding to HIV-1 virions and of gp120 dissociation. *AIDS Res. Hum. Retroviruses* **8**:443–50.

Moore, J. P., McKeating, J. A., Huang, Y. X., Ashkenazi, A., and Ho, D. D. (1992). Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. *J. Virol.* **66**:235–43.

Moore, J. P., McKeating, J. A., Norton, W. A., and Sattentau, Q. J. (1991). Direct measurement of soluble CD4 binding to human immunodeficiency virus type 1 virions: gp120 dissociation and its implications for virus-cell binding and fusion reactions and their neutralization by soluble CD4. *J. Virol.* **65**:1133–40.

Moore, J. P., McKeating, J. A., Weiss, R. A., and Sattentau, Q. J. (1990). Dissociation of gp120 from HIV-1 virions induced by soluble CD4. *Science* **250**:1139–42.

Muster, T., Guinea, R., Trkola, A., Purtscher, M., Klima, A., Steindl, F., Palese, P., and Katinger, H. (1994). Cross-neutralizing activity against divergent human immunodeficiency virus type 1 isolates induced by the gp41 sequence ELDKWAS. *J. Virol.* **68**:4031–4.

Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Ruker, F., and Katinger, H. (1993). A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. *J. Virol.* **67**:6642–7.

Neurath, A. R., Strick, N., Lin, K., and Jiang, S. (1995). Multifaceted consequences of anti-gp41 monoclonal antibody 2F5 binding to HIV type 1 virions. *AIDS Res. Hum. Retroviruses* **11**:687–96.

Oldstone, M. B., Tishon, A., Lewicki, H., Dyson, H. J., Feher, V. A., Assa-Munt, N., and Wright, P. E. (1991). Mapping the anatomy of the immunodominant domain of the human immunodeficiency virus gp41 transmembrane protein: peptide conformation analysis using monoclonal antibodies and proton nuclear magnetic resonance spectroscopy. *J. Virol*. **65**:1727–34.

Owens, R. J., Burke, C., and Rose, J. K. (1994). Mutations in the membrane-spanning domain of the human immunodeficiency virus envelope glycoprotein that affect fusion activity. *J. Virol*. **68**:570–4.

Owens, R. J., 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**:3987–3991.

Pinter, A., Honnen, W. J., Tilley, S. A., Bona, C., Zaghouani, H., Gorny, M. K., and Zolla-Pazner, S. (1989). Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. *J. Virol*. **63**:2674–2679.

Purtscher, M., Trkola, A., Grassauer, A., Schulz, P. M., Klima, A., Dopper, S., Gruber, G., Buchacher, A., Muster, T., and Katinger, H. (1996). Restricted antigenic variability of the epitope recognized by the neutralizing gp41 antibody 2F5. *AIDS* **10**:587–93.

Radding, W., Pan, Z. Q., Hunter, E., Johnston, P., Williams, J. P., and McDonald, J. M. (1996). Expression of HIV-1 envelope glycoprotein alters cellular calmodulin. *Biochem. Biophys. Res. Comm*. **218**:192–197.

Robinson, M. S., Watts, C., and Zerial, M. (1996). Membrane dynamics in endocytosis. *Cell* **84**:13–21.

Robinson, W., Jr., Kawamura, T., Gorny, M. K., Lake, D., Xu, J. Y., Matsumoto, Y., Sugano, T., Masuho, Y., Mitchell, W. M., Hersh, E., et al. (1990a). Human monoclonal antibodies to the human immunodeficiency virus type 1 (HIV-1) transmembrane glycoprotein gp41 enhance HIV-1 infection in vitro. *Proc. Natl. Acad. Sci. USA* **87**:3185–9.

Robinson, W., Jr., Kawamura, T., Lake, D., Masuho, Y., Mitchell, W. M., and Hersh, E. M. (1990b). Antibodies to the primary immunodominant domain of human immunodeficiency virus type 1 (HIV-1) glycoprotein gp41 enhance HIV-1 infection in vitro. *J. Virol*. **64**:5301–5.

Roth, M. G., Srinivas, R. V., and Compans, R. W. (1983). Basolateral maturation of retroviruses in polarized epithelial cells. *J. Virol*. **45**:1065–73.

Rowell, J. F., Stanhope, P. E., and Siliciano, R. F. (1995). Endocytosis of endogenously synthesized HIV-1 envelope protein. Mechanism and role in processing for association with class II MHC. *J. Immunol*. **155**:473–88.

Saag, M., Alldredge, L., Kilby, M., et al. (1997). A short term assessment of the safety, pharmacokinetics, and antiviral activity of T-20: An inhibitor of gp41 mediated membrane fusion. 35th Meeting of the IDSA, pp. Abstract #771.

Salzwedel, K., Johnston, P. B., Roberts, S. J., Dubay, J. W., and Hunter, E. (1993). Expression and characterization of glycophospholipid-anchored human immunodeficiency virus type 1 envelope glycoproteins. *J. Virol*. **67**:5279–88.

Sattentau, Q. J., and Moore, J. P. (1991). Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. *J. Exp. Med*. **174**:407–15.

Sattentau, Q. J., and Moore, J. P. (1993). The role of CD4 in HIV binding and entry. *Philos. Trans. R. Soc. Lond. B. Biol. Sci*. **342**:59–66.

Sattentau, Q. J., Moore, J. P., Vignaux, F., Traincard, F., and Poignard, P. (1993). Conformational changes induced in the envelope glycoproteins of the human and simian immunodeficiency viruses by soluble receptor binding. *J. Virol*. **67**:7383–93.

Sattentau, Q. J., Zolla-Pazner, S., and Poignard, P. (1995). Epitope exposure on functional, oligomeric HIV-1 gp41 molecules. *Virology* **206**:713–7.

Sauter, M. M., Pelchen-Matthews, A., Bron, R., Marsh, M., LaBranche, C. C., Vance, P. J., Romano, J., Haggarty, B. S., Hart, T. K., Lee, W. M., and Hoxie, J. A. (1996). An internalization signal in the

simian immunodeficiency virus transmembrane protein cytoplasmic domain modulates expression of envelope glycoproteins on the cell surface. *J Cell Biol* **132**:795–811.

Schawaller, M., Smith, G. E., Skehel, J. J., and Wiley, D. C. (1989). Studies with crosslinking reagents on the oligomeric structure of the env glycoprotein of HIV. *Virology* **172**:367–369.

Sekigawa, I., Chamow, S. M., Groopman, J. E., and Byrn, R. A. (1990). CD4 immunoadhesin, but not recombinant soluble CD4, blocks syncytium formation by human immunodeficiency virus type 2-infected lymphoid cells. *J. Virol.* **64**:5194–8.

Shankar, P., Fabry, J. A., Fong, D. M., and Lieberman, J. (1996). Three regions of HIV-1 gp160 contain clusters of immunodominant CTL epitopes. *Immunol. Lett.* **52**:23–30.

Shugars, D. C., Wild, C. T., Greenwell, T. K., and Matthews, T. J. (1996). Biophysical characterization of recombinant proteins expressing the leucine zipper-like domain of the human immunodeficiency virus type 1 transmembrane protein gp41. *J. Virol.* **70**:2982–91.

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**:88–92.

Skehel, J. J., Bayley, P. M., Brown, E. B., Martin, S. R., Waterfield, M. D., White, J. M., Wilson, I. A., and Wiley, D. C. (1982). Changes in the conformation of influenza virus hemagglutinin at the pH optimum of virus-mediated membrane fusion. *Proc. Natl. Acad. Sci. USA* **79**:968–72.

Srinivas, S. K., Srinivas, R. V., Anantharamaiah, G. M., Compans, R. W., and Segrest, J. P. (1993). Cytosolic domain of the human immunodeficiency virus envelope glycoproteins binds to calmodulin and inhibits calmodulin-regulated proteins. *J Biol Chem* **268**:22895–9.

Steffy, K. R., Kraus, G., Looney, D. J., and Wong-Staal, F. (1992). Role of the fusogenic peptide sequence in syncytium induction and infectivity of human immunodeficiency virus type 2. *J. Virol.* **66**:4532–5.

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**:659–68.

Sullivan, N., Sun, Y., Li, J., Hofmann, W., and Sodroski, J. (1995). Replicative function and neutralization sensitivity of envelope glycoproteins from primary and T-cell line-passaged human immunodeficiency virus type 1 isolates. *J. Virol.* **69**:4413–22.

Tencza, S. B., Mietzner, T. A., and Montelaro, R. C. (1997). Calmodulin-binding function of LLP segments from the HIV type 1 transmembrane protein is conserved among natural sequence variants. *AIDS Res. Hum. Retroviruses* **13**:263–9.

Tencza, S. B., Miller, M. A., Islam, K., Mietzner, T. A., and Montelaro, R. C. (1995). Effect of amino acid substitutions on calmodulin binding and cytolytic properties of the LLP-1 peptide segment of human immunodeficiency virus type 1 transmembrane protein. *J. Virol.* **69**:5199–202.

Trowbridge, I. S., Collawn, J. F., and Hopkins, C. R. (1993). Signal-dependent membrane protein trafficking in the endocytic pathway. *Annu. Rev. Cell. Biol.* **9**:129–61.

Vincent, M. J., Raja, N. U., and Jabbar, M. A. (1993). Human immunodeficiency virus type 1 Vpu protein induces degradation of chimeric envelope glycoproteins bearing the cytoplasmic and anchor domains of CD4: role of the cytoplasmic domain in Vpu-induced degradation in the endoplasmic reticulum. *J. Virol.* **67**:5538–49.

Weiss, C. D., Levy, J. A., and White, J. M. (1990). Oligomeric organization of gp120 on infectious human immunodeficiency virus type 1 particles. *J. Virol.* **64**:5674–5677.

Weiss, C. D., and White, J. M. (1993). Characterization of stable Chinese hamster ovary cells expressing wild-type, secreted, and glycosylphosphatidylinositol-anchored human immunodeficiency virus type 1 envelope glycoprotein. *J. Virol.* **67**:7060–6.

Weissenhorn, W., Dessen, A., Harrison, S. C., Skehel, J. J., and Wiley, D. C. (1997). Atomic structure of the ectodomain from HIV-1 gp41. *Nature* **387**:426–30.

Weissenhorn, W., Wharton, S. A., Calder, L. J., Earl, P. L., Moss, B., Aliprandis, E., Skehel, J. J., and Wiley, D. C. (1996). The ectodomain of HIV-1 env subunit gp41 forms a soluble, alpha- helical, rod-like oligomer in the absence of gp120 and the N-terminal fusion peptide. *EMBO J*. **15**:1507–14.

Werner, A., Winskowsky, G., and Kurth, R. (1990). Soluble CD4 enhances simian immunodeficiency virus SIVagm infection. *J. Virol*. **64**:6252–6.

White, J., Kielian, M., and Helenius, A. (1983). Membrane fusion proteins of enveloped animal viruses. *Q. Rev. Biophys*. **16**:151–95.

White, J. M. (1992). Membrane fusion. *Science* **258**:917–24.

Wild, C., Dubay, J. W., Greenwell, T., Baird, T., Jr., Oas, T. G., McDanal, C., Hunter, E., and Matthews, T. (1994b). Propensity for a leucine zipper-like domain of human immunodeficiency virus type 1 gp41 to form oligomers correlates with a role in virus- induced fusion rather than assembly of the glycoprotein complex. *Proc. Natl. Acad. Sci. USA* **91**:12676–80.

Wild, C., Greenwell, T., and Matthews, T. (1993). A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus- mediated cell-cell fusion. *AIDS Res. Hum. Retroviruses* **9**:1051–3.

Wild, C., Greenwell, T., Shugars, D., Rimsky-Clarke, L., and Matthews, T. (1995). The inhibitory activity of an HIV type 1 peptide correlates with its ability to interact with a leucine zipper structure. *AIDS Res. Hum. Retroviruses* **11**:323–5.

Wild, C., Oas, T., McDanal, C., Bolognesi, D., and Matthews, T. (1992). A synthetic peptide inhibitor of human immunodeficiency virus replication: correlation between solution structure and viral inhibition. *Proc. Natl. Acad. Sci. USA* **89**:10537–41.

Wild, C. T., Shugars, D. C., Greenwell, T. K., McDanal, C. B., and Matthews, T. J. (1994a). Peptides corresponding to a predictive alpha-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection. *Proc. Natl. Acad. Sci. USA* **91**:9770–4.

Wilk, T., Pfeiffer, T., and Bosch, V. (1992). Retained in vitro infectivity and cytopathogenicity of HIV-1 despite truncation of the C-terminal tail of the env gene product. *Virology* **189**:167–77.

Wilk, T., Pfeiffer, T., Bukovsky, A., Moldenhauer, G., and Bosch, V. (1996). Glycoprotein incorporation and HIV-1 infectivity despite exchange of the gp160 membrane-spanning domain. *Virology* **218**:269–74.

Wilson, I. A., Skehel, J. J., and Wiley, D. C. (1981). Structure of the haemagglutinin membrane glycoprotein of influenza virus at 3 Å resolution. *Nature* (London) **289**:366–373.

Xu, J. Y., Gorny, M. K., Palker, T., Karwowska, S., and Zolla-Pazner, S. (1991). Epitope mapping of two immunodominant domains of gp41, the transmembrane protein of human immunodeficiency virus type 1, using ten human monoclonal antibodies. *J. Virol*. **65**:4832–8.

Zingler, K., and Littman, D. R. (1993). Truncation of the cytoplasmic domain of the simian immunodeficiency virus envelope glycoprotein increases Env incorporation into particles and fusogenicity and infectivity. *J. Virol*. **66**:2824–2831.