Charon's temperature would be near 58 K. The vapor pressure of methane rises exponentially with temperature in this regime, from 3.5 μbar at 50 K to 59 μbar at 58 K (*14*). Since the root-mean-square thermal velocity of methane is about half of the escape velocity from Charon to infinity, and an even greater fraction of the velocity required for transfer through the inner Lagrange point onto Pluto, it is easy to show that Charon's inventory of methane would be lost on time scales short compared to the age of the solar system, whether by Jeans escape or hydrodynamic blowoff (*15*).

The details of the partitioning of methane between escape to infinity and transfer onto Pluto are as yet unclear, but escape of up to 22 km of methane from Charon can occur over the age of the solar system. After shedding several kilometers of methane, the surface of Charon would be expected to resemble a global "moraine," with the residuum composed of (cosmically abundant) water ice and a "slag" of darker carbonaceous or silicaceous impurities or both. This process could explain both the compositional difference and also why Charon's visual albedo is significantly less than that of Pluto (*16*).

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Functional Regions of the Envelope Glycoprotein of Human Immunodeficiency Virus Type 1

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The envelope of the human immunodeficiency virus type 1 (HIV-1) plays a central role in the process of virus entry into the host cell and in the cytopathicity of the virus for lymphocytes bearing the CD4 molecule. Mutations that affect the ability of the envelope glycoprotein to form syncytia in CD4$^+$ cells can be divided into five groups: those that decrease the binding of the envelope protein to the CD4 molecule, those that prevent a post-binding fusion reaction, those that disrupt the anchorage of the envelope glycoprotein in the membrane, those that affect the association of the two subunits of the envelope glycoprotein, and those that affect post-translational proteolytic processing of the envelope precursor protein. These findings provide a functional model of the HIV envelope glycoprotein.

THE HUMAN IMMUNODEFICIENCY virus type 1 (HIV-1), also called HTLV-III or LAV-1, is the etiological agent of the acquired immune deficiency syndrome (AIDS) and related disorders (*1*). The viral envelope is synthesized as a 160-kilodalton (kD) (gp160) precursor glycoprotein, which is subsequently cleaved into 120-kD (gp120) and 41-kD (gp41) glycoproteins present on the virion particle (*2*). The gp120 exterior glycoprotein binds to the CD4 protein present on the surface of helper T lymphocytes, macrophages, and other cells (*3*), thus determining the tissue selectivity of viral infection. By analogy with other enveloped viruses, after the gp120 binds to CD4, virus entry is facilitated by an envelope-mediated fusion of the viral and target cell membranes.

The HIV-1 envelope glycoprotein is also responsible for at least some of the cytopathic effects of virus infection on CD4$^+$ cells in culture (*4, 5*). Expression of the envelope glycoprotein on the surface of infected cells mediates fusion events among CD4$^+$ cells via a reaction similar to that by which the virus enters the uninfected cell, leading to the formation of short-lived multinucleated giant cells. Syncytium formation is dependent on a direct interaction of the HIV-1 envelope with the CD4 protein (*3–5*).

To define the relation between the structure of the HIV-1 envelope glycoprotein and the ability to form syncytia, we introduced deletion and insertion mutations into a plasmid, pIIIenv3, that encodes the envelope glycoprotein derived from the HTLV-III$_B$ strain of HIV-1. The gene was present on a plasmid that also encodes the *art* gene product (*4, 6*). CD4$^+$ and CD4$^-$ cell lines that expressed the HIV-1 *tat* gene product constitutively (*7*) were used as recipients in a transient transfection assay. To determine the size of the cell-associated and released proteins, we conducted radioimmunoprecipitation studies using antisera from AIDS patients or a goat antiserum to gp120 (*8*) and detergent-disrupted cells or cell-free supernatants prepared 48 to 72 hours after the cells had been transfected with pIIIenv3. The ability of the envelope proteins to bind to the CD4 protein and to induce syncytia was also examined.

The integral membrane protein (gp41) of HIV-1 differs from that of most retroviruses in the presence of additional sequences at the carboxyl terminus (*9*). The gp41 on the carboxyl-terminal side of the probable membrane-spanning region consists of a hydrophilic region (residues 724–745) and a terminal region (residues 745–856) of alternating hydrophilic and hydrophobic character (Fig. 1). Large deletions of either of these regions resulted in mutant *env* proteins that efficiently formed syncytia [see plasmids pIIIenvΔ(727–751), Δ813, and Δ753]. However, deletion of both of these regions (pIIIenvΔ727) resulted in very low levels of *env* protein production and loss of syncytium formation. When the deleted sequences were replaced by sequences derived from the *art* gene or by random sequences that have varying degrees of hydrophobic or hydrophilic character, syncytium induction was observed (for example, pIIIenvΔ722S, Δ725S, and Δ732S). The *art* protein-derived amino acid sequence could be introduced in the amino-terminal direction up to amino acid 705 without eliminating the ability of the mutated *env* protein to yield syncytia (pIIIenvΔ705S). However, substi-

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tution of the identical amino acid sequences on the amino-terminal side of residue 705 (for example, the products encoded by pIIIenvΔ700S or pIIIenvΔ697S) resulted in undetectable levels of cell-associated *env* protein and lack of syncytium formation. These studies indicate that gp41 sequences on the carboxyl-terminal side of residue 705 are not necessary for the functions of the envelope involved in the formation of syncytia.

To examine the effect of deletion of the probable membrane-spanning region of gp41 (residues 666–722), we introduced translation termination codons in positions corresponding to amino acids 472, 517, 641, and 665 (pIIIenvΔ472S, Δ517, Δ641, and Δ665) (Fig. 1). Additionally, a series of polar amino acids were introduced into the region between amino acids 666 and 722 (pIIIenvΔ705S, Δ700S, and Δ697S). The level of cell-associated *env* products was markedly reduced for all of these mutants compared with the wild-type envelope. However, *env*-derived proteins were readily detected in the supernatants of the transfected cells (Fig. 1, B and C). The soluble products of pIIIenvΔ641, pIIIenvΔ700S, and pIIIenvΔ697S were larger than the wild-type gp120 or the soluble products of pIIIenvΔ472S or pIIIenvΔ517, suggesting that the former products are not processed by cleavage at the gp120–gp41 junction. The soluble products of the pIIIenvΔ665 and pIIIenvΔ705S appear to be partially processed, as some of the protein comigrates with wild-type gp120 whereas the remainder migrates more slowly on SDS-polyacrylamide gels.

The mutant *env* glycoproteins lacking the transmembrane region did not induce the formation of syncytia in $CD4^+$ lymphocytes. To examine whether the *env* products are capable of binding to the CD4 molecule, metabolically labeled supernatants from transfected cells were incubated with $CD4^+$ SupT1 lymphocytes (*10*). The SupT1 cells were then washed, lysed, and radioimmunoprecipitated with goat antiserum to gp120 (*8*). Envelope proteins bound to the SupT1 cells were identified on SDS-polyacrylamide gels. In parallel assays, lentil-lectin purified gp120, but not gp160, derived from HIV-1–infected cultures bound to the SupT1 cells. No binding was observed if $CD4^-$ Raji cells were used instead of the SupT1 cells. The envelope products encoded by pIIIenvΔ517 and pIIIenvΔ641 bound to the $CD4^+$ SupT1 lymphocytes in this assay (Fig. 2, A and B). The specificity of the binding reaction was ensured by incubating the labeled *env* products with the SupT1 lymphocytes in the presence of monoclonal

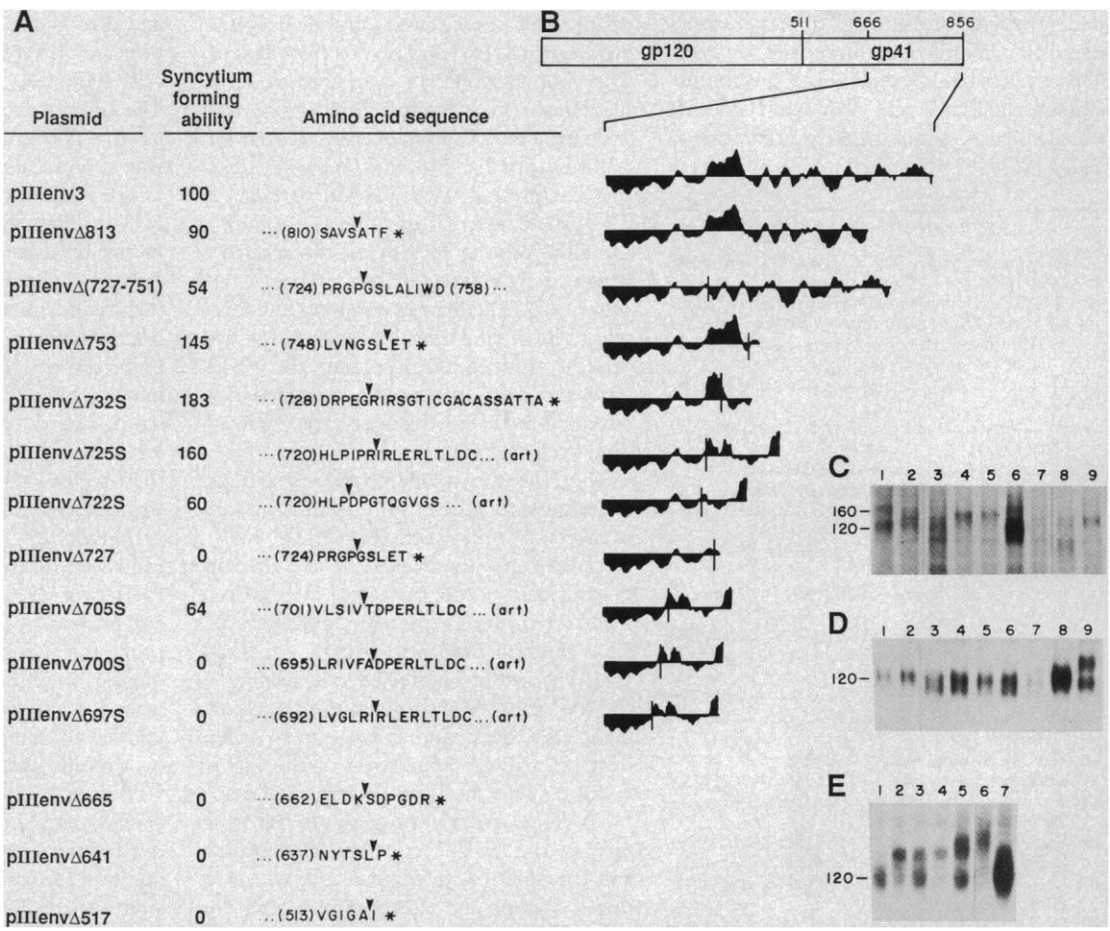

Fig. 1. Mutant envelopes with carboxy-terminal deletions. (**A**) Plasmids encoding *env* proteins were made by digestion of pIIIenv3 with Bam HI [nucleotide 8053 of the sequence of Ratner *et al.* (*9*)], digestion with Bal 31 exonuclease for timed periods, ligation of Bam HI 8-bp linkers (New England Biolabs), digestion with Bam HI, and re-ligation prior to transformation of *Escherichia coli*. The predicted structure of the mutant *env* products was determined by DNA sequence analysis. Plasmids are named according to the last wild-type amino acid residue remaining in the mutant *env* product; S indicates that a significant substitution of amino acids carboxyl terminal to the deletion has been made. Black arrows indicate the junction between wild-type and mutated sequences. Asterisks indicate the carboxyl terminus of the mutant envelope; "art" indicates that the mutation results in a frameshift of the *env* reading frame into that encoding the *art* protein. We made pIIIenvΔ641, pIIIenvΔ517, and pIIIenvΔ472S by introducing either stop codons or frameshifts into the *env* gene sequence near the indicated residues, by deletion of the 1474-bp Hind III fragment in the case of pIIIenvΔ641 or by insertion of synthetic linkers in the case of the other two plasmids. The amino acid sequence of the pIIIenvΔ472S product is (472)GAGIPEI*. Syncytium forming ability in transfected Jurkat-tat_{III} lymphocytes relative to that after transfection with pIIIenv3 is shown. (**B**) Predicted hydrophilic (above) and hydrophobic (below) patterns of the carboxyl-terminal regions of the mutant envelopes (*21*). Vertical lines indicate the junction between wild-type and mutated amino acid sequences. (**C** to **E**) The plasmids encoding mutant envelopes were transfected with a plasmid that expresses the HIV *art* protein (*6*). Radioimmunoprecipitates of transfected Raji-tat_{III} cells are shown for the cell lysates (C) or cell supernatants (D). In (C) and (D) the lanes were: 1, pIIIenv3; 2, pIIIenvΔ(727–751); 3, pIIIenvΔ753; 4, pIIIenvΔ732S; 5, pIIIenvΔ722S; 6, pIIIenvΔ725S; 7, pIIIenvΔ727; 8, pIIIenvΔ517; and 9, pIIIenvΔ665. In (E), which shows transfected Raji-tat_{III} cell supernatants, the lanes were 1, pIIIenv3; 2, pIIIenvΔ700S; 3, pIIIenvΔ705S; 4, pIIIenvΔ697S; 5, pIIIenvΔ665; 6, pIIIenvΔ641; and 7, pIIIenvΔ517.

antibodies to CD4 epitopes. The OKT4A but not the OKT4 monoclonal antibody inhibited the binding of labeled *env* products encoded by pIIIenvΔ517 and pIIIenvΔ641, as well as the binding of gp120 purified from HIV-1–infected cultures. Therefore, the inability of the pIIIenvΔ517 and pIIIenvΔ641 products to form syncytia was not due to an inability to bind to the CD4 molecule.

By contrast, an *env* product with a deletion of 39 amino acids from the carboxyl terminus of gp120 (made by plasmid pIIIenvΔ472S) did not bind to the $CD4^+$ SupT1 cells (Fig. 2C, lanes 9 and 21). Thus, the addition of 6 or 130 amino acids derived from gp41 did not eliminate the ability of the exterior envelope protein to bind to the CD4 molecule, whereas a deletion near the carboxyl terminus of gp120 disrupted CD4 binding.

A series of four or five amino acid in-frame insertion mutants were created throughout the *env* gene (Table 1). One set of these mutants (pIIIenv103, 252, 287, 342, and 448) that fail to form syncytia upon transfection of $CD4^+$ cells was defective in the synthesis of gp120 as determined by immunoprecipitation of the labeled cell lysates or of cell-free supernatants (Fig. 3). However, cell-associated gp160 was produced in near normal amounts by these mutants. The gp160 protein had the same electrophoretic mobility as that produced by the wild-type *env* gene. Moreover, digestion of the wild-type and mutant gp160 proteins with endoglycosidase H yielded in both cases an 88-kD protein, indicating that both gp160 species contained similar amounts of high mannose sugar moieties. Evidently, these small insertions either prevent processing of gp160 or result in the production of a gp120 protein that has a very short half-life.

Two sets of insertion mutations that eliminate syncytium formation dramatically reduce the amount of gp120 associated with the cell yet produce abundant gp120 in the supernatant. One set of these mutants is located in the amino-terminal half of gp120 (pIIIenv65, 129, 174, 204, and 308) and the second set is located in the amino-terminal half of gp41 (pIIIenv530, 537, 640A, and 640B). The amount of cell-associated gp160 produced by these mutants is near normal. These mutations apparently disrupt the association of the gp120 and gp41 *env* glycoproteins.

Two approaches were taken to detect mutations that affected the binding of gp120 to the CD4 protein. If a substantial amount of the mutant gp120 protein was shed into the medium of transfected cells, the metabolically labeled supernatants were incubated with $CD4^+$ lymphocytes, which were then washed, lysed, and radioimmunoprecipitated with goat antiserum to gp120 (Table 1 and Fig. 2). For a number of the mutants, release of the altered *env* product into the supernatant was enhanced by dele-

Table 1. Linker insertion mutations in the HIV-1 envelope. The plasmids encoding the mutant *env* proteins were made by partial digestion of pIIIenv3 with restriction enzymes (Rsa I, Mnl I, Dra I, Nsi I, Stu I, Pvu II, Alu I, or Bbv I), creation of blunt ends using the Klenow fragment of DNA polymerase I or T4 DNA polymerase, if necessary, and ligation of appropriately sized synthetic linkers (New England Biolabs) to preserve the envelope reading frame. After digestion of the synthetic linkers, the singly cut DNA was isolated on a low-melting point agarose gel, ligated, and used to transform *E. coli*. The position of the linker insert was analyzed by restriction enzyme digestion and DNA sequence analysis (*17*). The predicted amino acid sequence of the expressed *env* product is compared to the sequence of the wild-type *env* product; the residue number in parentheses refers to the amino-terminal amino acid shown, based on the sequence of the HXBc2 infectious provirus we used (*9, 18*). The CsCl-purified plasmids were transfected by the DEAE-dextran procedure (*19*) into Jurkat-tat_{III} and Raji-tat_{III} lymphocytes (*7*), which were labeled with [^{35}S]cysteine or scored for syncytia in the case of the Jurkat-tat_{III} transfectants. Labeled cell lysates and supernatants were radioimmunoprecipitated (*20*) with RV119 AIDS patient serum or with goat antiserum to gp120 (*8*). The level of *env* products in cell lysates or supernatants is indicated. Syncytium formation is the percentage of number of syncytia observed after transfection of equal numbers of Jurkat-tat_{III} cells with the mutated plasmid as compared with pIIIenv3. CD4 binding was determined by using the gp120 in the supernatant of transfected cells ("gp120" column) or a plasmid containing the indicated linker insertion plus a second mutation that results in the placement of a stop codon near the Hind III site at nucleotide 7720 ("Δ641" column). The stop codon in the Δ641 mutants results in the truncation of the *env* product amino terminal to the gp41 transmembrane region (see pIIIenvΔ641 in Fig. 1). Supernatants from transfected Raji-tat_{III} lymphocytes were labeled with [^{35}S]cysteine and incubated with approximately 10^7 SupT1 lymphocytes (*10*) in the presence of 1.0 μg/ml of OKT4 or OKT4A (Ortho) for 1 hour at 37°C. The SupT1 cells were centrifuged, washed in phosphate-buffered saline, lysed, and used for radioimmunoprecipitation with goat antiserum to gp120 or with AIDS patient serum. Labeled proteins that were not bound to the SupT1 cells were also immunoprecipitated. ND, not done; A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Plasmid	Predicted amino acid sequence	Cell-associated		Supernatant gp120	CD4 binding		Syncytium formation
		gp160	gp120		gp120	Δ641	
pIIIenv3	Wild-type	+	++	+	+	+	100
pIIIenv65	(64)GVH→GVDLDRH	+	±	+	+	ND	<1
pIIIenv103	(102)EQM→EHRNSGM	+	−	−	ND	ND	<1
pIIIenv129	(128)SLK→SFRNSGK	+	−	++	+	+	<1
pIIIenv174	(172)EYAF→EYHRWF	+	−	++	+	+	<1
pIIIenv204	(203)QAC→QAHRWAC	+	−	++	+	+	<1
pIIIenv252	(251)IRPV→IRRSRSPV	+	−	−	ND	ND	<1
pIIIenv287	(286)VQLN→VQPIDGLN	+	−	−	ND	ND	<1
pIIIenv308	(307)IRIQ→IRPELIPVQ	+	±	++	+	ND	<1
pIIIenv342	(341)TLKQ→TFRNSGK	+	−	−	ND	ND	<1
pIIIenv363	(362)KQSS→KQPELIPAS	+	++	+	−	ND	<1
pIIIenvΔ(394–401)	(392)NSTWFNSTWSTE→NSTE	+	++	+	+	ND	90
pIIIenv419	(418)CRI→CTGINSGI	Low	Low	Low	ND	−	<1
pIIIenv448	(447)SNI→SNGIPNI	+	−	−	ND	ND	<1
pIIIenv473A	(472)GGD→GAGINSGD	+	+	+	−	ND	<1
pIIIenv473B	(472)GGD→GAGINSIDGINSGD	+	+	+	−	ND	<1
pIIIenv517A	(516)GALF→GAGIPALF	+	++	+	+	ND	1-5
pIIIenv517B	(516)GALF→GAIHRWIALF	+	+	+	+	ND	<1
pIIIenv530	(529)TMG→TMEFPMG	+	−	+	ND	ND	<1
pIIIenv537	(536)TLT→TLEFPLT	+	−	+	ND	ND	<1
pIIIenv640A	(639)TSL→TSSSMSL	+	−	+	+	ND	<1
pIIIenv640B	(639)TSL→TSRNSGL	+	−	+	ND	ND	<1

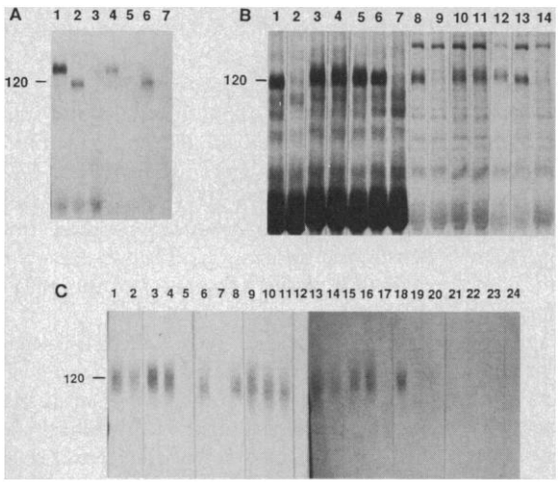

Fig. 2. CD4 binding ability of mutant envelopes. Labeled supernatants from transfected Raji-tat_{III} lymphocytes were tested for their ability to bind to the surface of SupT1 lymphocytes (see legend to Table 1). The radioimmunoprecipitates of proteins bound or not bound to the SupT1 cells are shown. (**A**) Lanes 1 to 3, unbound precipitates; lanes 4 to 7, bound precipitates. Lanes 1, 4, and 5, pIIIenvΔ641; lanes 2, 6, and 7, pIIIenv3; lane 3, pBR322. SupT1 cells were incubated with OKT4 in lanes 4 and 6 or with OKT4A in lanes 5 and 7. (**B**) Lanes 1 to 7, unbound precipitates; lanes 8 to 14, bound precipitates. Lanes 1 and 8, pIIIenv3; lanes 2 and 9, pIIIenv103; lanes 3 and 10, pIIIenv129; lanes 4 and 11, pIIIenv174; lanes 5 and 12, pIIIenv204; lanes 6 and 13, pIIIenv517A; lanes 7 and 14, pIIIenvFSB. pIIIenvFSB contains a frameshift mutation at the Bgl II site [nucleotide 8630 of the sequence of Ratner *et al.* (*9*)], within the gp120-coding sequences. (**C**) Lanes 1 to 12, unbound precipitates; lanes 13 to 24, bound precipitates. Lanes 1 and 13, pIIIenv3; lanes 2 and 14, pIIIenv65; lanes 3 and 15, pIIIenv129; lanes 4 and 16, pIIIenv204; lanes 5 and 17, pIIIenv252; lanes 6 and 18, pIIIenv308; lanes 7 and 19, pIIIenv342; lanes 8 and 20, pIIIenv363; lanes 9 and 21, pIIIenvΔ472S; lanes 10 and 22, pIIIenv473A; lanes 11 and 23, pIIIenv473B; lanes 12 and 24, pBR322.

tion of the transmembrane region. The ability of the pIIIenvΔ641 protein and mutant derivatives to bind to the CD4 molecule was assessed in a manner similar to that described above. In both instances, an *env* product lacking mutations within the gp120-coding sequences was capable of binding to $CD4^+$ lymphocytes (Fig. 2A). The binding of wild-type and mutant *env* proteins to $CD4^+$ lymphocytes was inhibited by OKT4A but not OKT4 monoclonal antibodies.

Mutations within *env* gene sequences encoding the amino-terminal half of gp120 do not disrupt the ability of the *env* product to specifically bind to $CD4^+$ SupT1 cells (Fig. 2, B and C, and Table 1). By contrast, the *env* products with changes in the carboxyl-terminal half of gp120 (pIIIenv363, 419, 473A, and 473B) did not efficiently bind to $CD4^+$ lymphocytes. In the case of pIIIenv363, 473A, and 473B, although the mutations did not apparently affect the synthesis, processing, and cell association of the gp160 and gp120 products, no syncytia were induced by the altered *env* proteins. Deletion of the 39 amino acids at the carboxyl terminus of gp120 also yielded a protein that did not bind to CD4 (see above). An eight–amino acid deletion located between residues 394 and 401 of gp120 did not affect either CD4 binding or syncytium forming ability [see pIIIenvΔ(394–401) in Table 1].

After binding to the CD4 molecule, the HIV envelope induces fusion of cell membranes. The plasmids pIIIenv517A and pIIIenv517B contain mutations in the region encoding the hydrophobic amino terminus of gp41. Although the level of cell-associated gp120 produced by pIIIenv517B was slightly reduced compared to that of the wild-type plasmid, the *env* products produced by pIIIenv517A were indistinguishable from wild-type products (Fig. 3). In both instances, the gp120 protein was capable of binding specifically to the surface of $CD4^+$ lymphocytes (Fig. 2B). Nonetheless, the mutant envelopes produced by pIIIenv517A and pIIIenv517B were markedly compromised in their ability to induce syncytia in $CD4^+$ lymphocytes compared with the wild-type *env* proteins (Table 1). Apparently a function critical for syncytium formation following the binding of gp120 to CD4 is affected by the mutations in pIIIenv517A and pIIIenv517B.

Mutations that affect the ability of the HIV-1 *env* glycoprotein to induce syncytia in $CD4^+$ cells may be divided into five groups: those that disrupt the binding of CD4 by gp120, those that affect a post-binding fusion event, those that affect the membrane association of the gp120-gp41 complex, those that affect the association of the gp120 and gp41 proteins, and those that disrupt the processing of gp160 to mature envelope proteins. Other mutations have no discernible phenotype either with respect to syncytium formation or the production and processing of the *env* gene products. The location of the mutations relative to amino acid sequences conserved among HIV-1 isolates (*11*) and among HIV-1, HIV-2, and STLV-III (*12*) is shown in Fig. 4.

The regions defined by mutagenesis to be important for CD4 binding are located in the carboxyl-terminal portion of gp120, in amino acid sequences that are well conserved among HIV isolates and among related simian and human viruses (*11, 12*). The regions demonstrated herein to be important for CD4 binding are flanked by three regions that are highly variable among HIV isolates, at least one of which can be mutated without affecting either CD4 binding ability or syncytium formation [pIIIenvΔ(394–401) in Table 1]. The observations that denaturation of gp120 eliminates CD4 binding ability (*13*) and that conserved regions critical for CD4 binding are interspersed with hypervariable regions suggest that the gp120 sequences that interact directly with the CD4 molecule are discontinuous and are dependent on tertiary structural determinants for proper alignment.

Mutations in sequences that encode the hydrophobic amino terminus of the gp41 transmembrane protein interfere with a post-CD4 binding event in the process of membrane fusion. In other enveloped viruses, separate envelope proteins are involved in the binding and fusion reactions; membrane fusion is a function of the hydrophobic amino terminus of an integral membrane protein that undergoes maturation by proteolytic cleavage (*14*). Our results suggest that for HIV-1, similar assignment of binding functions to the gp120 protein and fusion functions to the gp41 protein is reasonable and that both functions are essential for syncytium formation. The cleavage of the gp160 precursor protein is likely to be important for both CD4 binding and membrane fusion. Lentil lectin-purified

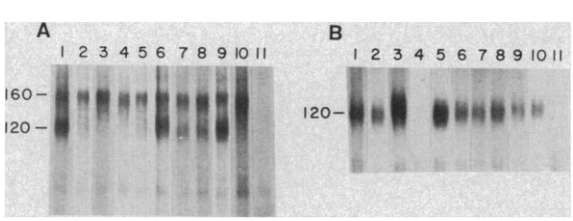

Fig. 3. Radioimmunoprecipitates of transfected Raji-tat_{III} lymphocytes. Raji-tat_{III} cells were transfected with envelope-expressing plasmids, labeled, and used for radioimmunoprecipitation (see legend to Table 1). Both cell lysates (**A**) and supernatants (**B**) were examined. Lane 1, pIIIenv3; lane 2, pIIIenv65; lane 3, pIIIenv129; lane 4, pIIIenv252; lane 5, pIIIenv308; lane 6, pIIIenv363; lane 7, pIIIenv473A; lane 8, pIIIenv517B; lane 9, pIIIenv517A; lane 10, pIIIenv640A; and lane 11, pBR322 DNA.

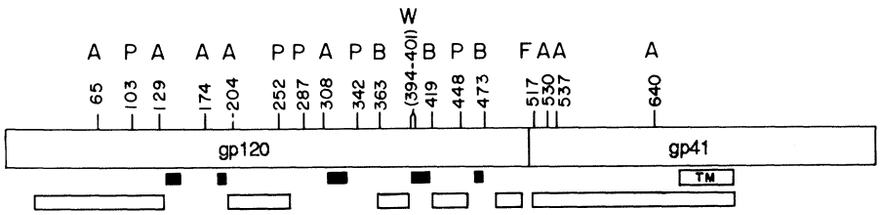

Fig. 4. Location and phenotypes of HIV-1 envelope mutations. The positions of the linker insertion mutations (numbers correspond to amino acid residues) are shown on a linear diagram of the gp120-gp41 envelope proteins. The positions of regions conserved among HIV-1, HIV-2, and STLV-III (open boxes), hypervariable regions (dark boxes), and the transmembrane region (TM) are depicted. The envelope function altered for each mutant is also shown (P, processing of gp160; A, association of gp120 and gp41 proteins; B, gp120-CD4 binding; F, membrane fusion events following gp120-CD4 binding; W, wild type).

gp120 binds specifically to the surface of $CD4^+$ cells much more efficiently than does similarly purified gp160. Likewise, exposure of the hydrophobic amino terminus of gp41, by analogy with the fusion proteins of orthomyxoviruses and paramyxoviruses, is probably required for initiation of membrane fusion (*14*).

A substantial amount of gp120 is released into the supernatants of cells expressing the wild-type *env* glycoprotein. The relative amount of gp160 and gp120 product observed after immunoprecipitation of HIV-1–infected cell lysates is not affected by the presence or absence of agents that reduce disulfide bonds (*13, 15*). These observations indicate that gp120 is not covalently bound by disulfide bonds to gp41. The clustering of mutations affecting gp120-gp41 association suggests that the amino terminus of gp120 makes multiple contacts with the exterior portion of gp41 and that these contacts are responsible for the association of gp120 with gp41 after proteolytic cleavage.

Large deletions in the carboxyl-terminus region of gp41, although exhibiting complex effects on levels of cell-associated envelope products, do not destroy the ability of the envelope protein to induce syncytia. However, mutations in this region that do not appreciably affect syncytium formation markedly attenuate virus replication. Deletion of 43 amino acids from the carboxyl terminus of the gp41 protein results in a poorly replicating virus that retains cytopathic activity for $CD4^+$ lymphocytes (*16*). Evidently, the carboxyl terminus of gp41 is critical for virus replication but not for syncytium formation or cytopathicity.

Assuming that the major effect of most of the mutations is to disrupt the function of regions proximal to the inserted amino acids, we can compose a functional model of the HIV-1 envelope. This assumption is reasonable given the physical clustering of mutants with similar functional phenotypes. In this model, the HIV-1 envelope interacting with the CD4 glycoprotein forms a "molecular bridge" between the lipid membranes that will undergo fusion. The gp120 exterior glycoprotein acts as a bifunctional molecule: association with the gp41 transmembrane protein is determined by regions located in the amino-terminal half, whereas association with the CD4 receptor is determined by carboxyl-terminal regions. After formation of the molecular bridge, the mobility afforded by the noncovalent nature of the gp120-gp41 bond may allow the efficient exposure of the target cell membrane to the hydrophobic gp41 regions that mediate the fusion process.

A functional model for the HIV-1 envelope should provide a rational basis for the design of strategies aimed at interfering with the role of the envelope glycoprotein in virus entry and $CD4^+$ lymphocyte depletion.

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