Effect of Point Mutations in the N Terminus of the Lentivirus Lytic Peptide-1 Sequence of Human Immunodeficiency Virus Type 1 Transmembrane Protein gp41 on Env Stability*

Sheau-Fen Lee‡, Chiung-Yuan Ko‡, Chin-Tien Wang§, and Steve S.-L. Chen¶

From the ¶Division of Infectious Diseases, Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, §Institute of Clinical Medicine, National Yang-Ming University, School of Medicine, and Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei 11217, Taiwan, Republic of China

To understand the role of the lentivirus lytic peptide-1 region of the human immunodeficiency virus type 1 transmembrane glycoprotein (gp) 41 in viral infection, we examined the effects on virus replication of single amino acid deletions spanning this region in an infectious provirus of the HXB2 strain. Among the mutants analyzed, only the deletion of one of the two adjacent valine residues located at positions 832 and 833 (termed the Δ833 mutant for simplicity) greatly reduced the steady-state, cell-associated levels of the Env precursor and gp120, as opposed to the wild-type virus. The altered Env phenotype resulted in severely impaired virus infectivity and gp120 incorporation into this mutant virion. Analyses of additional mutants with deletions at Ile-830, Ala-836, and Ile-840 demonstrated that the Δ830 mutant exhibited the most significant inhibitory effect on Env steady-state expression. These results indicate that the N terminus of the lentivirus lytic peptide-1 region is critical for Env steady-state expression. Among the mutant viruses encoding Env proteins in which residues Val-832 and Val-833 were individually substituted by nonconserved amino acids Ala, Ser, or Pro, which were expected to disrupt the α-helical structure in the increasingly severe manner of Pro > Ser > Ala, only the 833P mutant exhibited significantly reduced steady-state Env expression. Pulse labeling and pulse-chase studies demonstrated that the Δ830, Δ833, and 833P mutants of Env proteins degraded more rapidly in a time-dependent manner after biosynthesis than did the wild-type Env. The results indicate that residue 830 and 833 mutations are likely to induce a conformational change in Env that targets the mutant protein for cellular degradation. Our study has implications about the structural determinants located at the N terminus of the lentivirus lytic peptide-1 sequence of gp41 that affect the fate of Env in virus-infected cells.

The cytoplasmic domain of the human immunodeficiency virus, type 1 (HIV-1),1 transmembrane (TM) protein gp41 has been implicated in several steps of the virus life cycle. The viral replication functions that are affected by large truncations, deletions, or mutations in this region include virus replication, infectivity, transmission, cytopathogenicity (1–8), viral uncoating, or penetration of the viral core into host cells (6), Env incorporation into virions (5, 7–10), and interactions with the viral matrix protein MA during virus assembly (11–16). In addition, the cytoplasmic tail has a role in regulation of Env expression on the cell surface (17, 18), basolateral targeting of virus budding in polarized cells (19, 20), and in interactions with cellular components such as induction of apoptosis (21, 22) and binding to the medium chain μ1 and μ2 subunits of the AP-2 and AP-1 clathrin-associated adaptor complexes (23, 24).

Although the gp41 cytoplasmic tail does not exhibit typical membrane-binding sequences, the HIV-1 isolates show remarkable conservation of amphipathic α-helical secondary structures. The segments 826–856, 770–795, and 786–812, referred to as lentivirus lytic peptides (LLP-1), LLP-2, and LLP-3, respectively, all exhibit features of an amphipathic α-helix (25–27). Peptides mimicking these sequences show strong interactions with membranes, resulting in perturbation of membrane permeability and increased bilayer conductance (28–33). The α-helical motifs of LLP-1 and LLP-2 have also been implicated in virus-mediated cytopathicity by binding to calmodulin (34–37), a critical mediator of secondary messenger calcium in cytoplasmic signal transduction cascades (38). Binding to these peptides thus inhibits calmodulin-dependent enzymes in vitro and phospholipid systems in T cells.

Our previous results (39) showed that an Env mutant that lacks the entire cytoplasmic tail is able to interfere in trans with wild-type (wt) virus infectivity through formation of a dysfunctional hetero-oligomer with the wt Env subunit. This cytoplasmic tail truncation mutant also exhibits dominant interference with virus transmission mediated by homologous and heterologous T-tropic isolates when the mutant env gene is targeted to human CD4+ cells (40). We further demonstrated that the C-terminal two-thirds segment of the gp41 cytoplasmic tail possesses the potential to self-assemble into a high ordered multimer and that this segment confers cellular membrane-binding ability (41, 42). The correlation of membrane-binding ability and the multimerization potential of subdomains of the cytoplasmic tail may have implications for understanding the role of the cytoplasmic tail in the virus life cycle.

The gp41 cytoplasmic tail may play another role besides...

* This work was supported by National Science Council Grants 90-2326-B-001-067 and the Institute of Biomedical Sciences, Academia Sinica, Republic of China. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶To whom correspondence should be addressed: Div. of Infectious Diseases, Institute of Biomedical Sciences, Academia Sinica, 128, Section 2, Yen-Chiu-Yuan Road, Taipei 11529, Taiwan, Republic of China. Tel.: 886-2-2652-3933; Fax: 886-2-2785-8847; E-mail: schen@ibms.sinica.edu.tw.

1 The abbreviations used are: HIV-1, human immunodeficiency virus, type 1; mAb, monoclonal antibody; TM, transmembrane; gp, glycoprotein; LLP, lentivirus lytic peptide; wt, wild type; HRP, horseradish peroxidase; LTR, long terminal repeat; HCMV, human cytomegalovirus virus; VSV, vesicular stomatitis virus; RT, reverse transcriptase; PBS, phosphate-buffered saline.
those of virus infectivity and transmission. Truncation of the last 147 amino acids from the C terminus of gp41, i.e. mutant TM709, significantly decreases Env stability, as judged by the greatly reduced Env steady-state level of this mutant compared with the wt virus (7). However, mutants bearing shorter truncations in the C-terminal cytoplasmic tail than in the TM709 mutant showed no effect on Env stability (7). In the context of the entire viral genome, Dubay et al. (5) demonstrated that removal of the last 19 amino acids, i.e. residues Arg-838 to Leu-856, reduces the stability of Env but that further deletions toward the TM region do not affect Env stability. This 19-aa amino acid deletion mutant shows no effect on Env stability when Env is expressed in the absence of other viral proteins (5). Gabuzda et al. (6) reported that in COS-1 cells, Env mutants with deletions from 814 to 856 and from 840 to 856 decrease the steady-state levels of mutant Env proteins compared with the steady-state level expressed by wt Env. In contrast, deletions of residues in the C-terminal to residue 846 do not affect steady-state Env expression (6). The observations of Dubay et al. (5) and Gabuzda et al. (6) suggest that the integrity of the cytoplasmic tail N-terminal to residue 846 is crucial, in a cell-type-dependent manner, for Env stability. However, the amino acid residues and/or or structural determinants located in the cytoplasmic tail critical for Env stability remain to be determined.

To define the role of the LLP-1 sequence in virus replication, in this study we introduced single point deletions and substitutions in the LLP-1 region and examined the effects of these mutations on virus replication. We found that a single deletion of one of the two adjacent valine residues located at 832 and 833 of Env (termed the Δ833 mutant for simplicity) greatly reduced Env steady-state expression compared with that of the wt virus. Studies of additional deletion mutants at Ile-830, Ala-836, and Ile-840 also showed that the N terminus of the LLP-1 motif is important in Env steady-state expression. Further analysis of amino acid specificity for Val-832 and Val-833 illustrated that Env steady-state expression could be altered by a single substitution of a Pro residue for Val-833. We also found that the Δ830, Δ833, and 833P mutant Env proteins were less stable than the wt Env after biosynthesis, presumably due to their transport to an intracellular site for degradation. This is the first demonstration to provide a basis for understanding the viral molecular determinants that modulate HIV-1 Env stability.

EXPERIMENTAL PROCEDURES

Cells and Hybridoma—293 and HeLa cells were cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum. CEM-SS and PM1 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Hybridoma Igg13, Chessie 8, 902, and 183 were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and injected intraperitoneally into BALB/c mice to produce ascitic fluids. Mouse monoclonal antibody (mAb) directed against an N-terminal peptide of β-actin was purchased from Sigma. Horseradish peroxidase (HRP)-linked sheep anti-mouse Ig and streptavidin-biotinylated HRP complex were obtained from Amersham Biosciences. Peroxidase-labeled affinity-purified rabbit anti-sheep IgG was purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD).

Mutagenesis and Construction of Plasmids—Single point deletions and substitutions as shown in Fig. 1 were introduced into the gp41 cytoplasmic tail-coding sequence of the HXB2R3 provirus (15) by oligonucleotide-directed, site-specific mutagenesis using a PCR overlap extension method (43, 44). The paired primers used in PCR encoding each point deletion in the gp41 C-terminal cytoplasmic tail were as follows: Δ818, 5'-GCTTTCGCTACCCCTGTCAGCAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); Δ836, 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (sense) and 5'-GCTGCTTGGCAATCTAGGCTACAGGCTACAGG-3' (antisense); Δ843, 5'-GCTTGGCGGCACTAGGCTACAGGCTACAGG-3' (antisense); Δ854, 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); Δ863, 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); Δ874, 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); Δ832, 5'-AAGCTGTAGAAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); 832P, 5'-GCTGGTCAGCCCTGTCAGCAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); 832P, 5'-GAAGCTACAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); 833S, 5'-GCTACTGCTATGGCGGCATTGAGCAAGCTAACAGC-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); 833P, 5'-GCTACTGCTATGGCGGCATTGAGCAAGCTAACAGC-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); 833P, 5'-GAAGCTACAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); 833P, 5'-GAAGCTACAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense); and 833P, 5'-GAAGCTACAGGCTACAGGCTACAGG-3' (sense) and 5'-ATAGAAGTACAGGCTACAGGCTACAGG-3' (antisense). One amplification used oligonucleotide 8423f 5'-GAAGAAGAAGGTG-CTGAGTTGT-3' (antisense); nucleotides positioned at 8423--8443 of the HXB2 strain of the provirus and the antisense oligonucleotide as specified for each mutation as the primers. The other amplification used 8433r 5'-GCTACTTGTGCCCTTGTGGTGAC-3' (antisense); nucleotides positioned at 8393 to 8396 of the HXB2 strain of the provirus and the sense oligonucleotide as specified for each mutation as the primers. The two PCR products obtained were then used as the templates, and oligonucleotides 8423f and 8393r were used as the primers in the second round of PCR. All PCRs were performed using Vent DNA polymerase (New England Biolabs, Beverly, MA) according to a PCR amplification program described previously (42). The PCR-amplified DNA fragments were ligated via the BamHI and Xho I sites into pGEM-7Zf (Promega Corp., Madison, WI). After confirmation of the insert sequences in the pGEM-7Zf constructs, the BamHI-HXol fragments were isolated and then inserted into the HXB2R3-TM844 provirus (7) at the corresponding sites. To obtain a wt HXB2R3 provirus, the BamHI-Xhol sequence in HXB2R3-TM844 was replaced by the homologous sequence derived from HXB2. To generate long terminal repeat (LTR)-containing expression plasmids, the KpnI-Xhol fragments isolated from mutant provirus plasmids were substituted for the homologous sequence in a version of wt pSV7-puro (40) in which the Xhol site located in the 5'-LTR was deleted. All mutant pGEM-7Zf, HXB2R3, and pSV7-puro constructs were autosequenced using 8423f as the primer to confirm the sequences of the mutated BamHI-Xhol fragments.

pCDNA3 (Invitrogen; Carlsbad, CA), a cytomegalovirus enhancer/promoter-driven plasmid that also encodes T7 and Sp6 promoters flanking the polylinking sites, was used to construct pCDNA3/wt env encoding the entire HIV-1 env gene in vitro transcription/translation. The BamHI site in pCDNA3 was first abolished by BamHI digestion followed by overhang filling using T4 DNA polymerase in the presence of dNTPs and by ligation using T4 DNA ligase. The full-length wt env gene was generated by PCR using wt pSV7-puro as the template, and 5'-CGAATTCTGGCGCGCCATCATGAGAGAGGAGATATCAGC-3' and 5'-GCTTGGCCTAGGAGAGGAGGAGATATCAGC-3' as the forward and reverse primers, respectively. The amplification was performed in Vent polymerase buffer with a final concentration of 4 mM MgSO4 and 5% dimethyl sulfoxide according to a PCR program described previously (41). The 2.6 kb EcoRI-Xhol fragment was then inserted into the corresponding sites in the BamHI-deleted pCDNA3 plasmid. The resultant pCDNA3/wt env plasmid encoded a Kozak sequence 5' to the ATG initiation codon of the Env protein, which was followed by the entire env coding sequence with a stop codon 5' to the Xhol recognition sequence. The entire env gene was confirmed by DNA sequencing. Mutant pSV7-puro plasmids encoding the Δ830, Δ833, or 833P mutants were generated by PCR using as template the wt HXB2R3 plasmid and the 0.38-kb KpnI-deleted BamHI-Xhol fragments. These fragments were then used to replace the homologous sequence in pCDNA3/wt env to yield various mutant pCDNA3/env plasmids. The mutant pCDNA3/env clones were also confirmed by DNA sequencing.

Plasmid DNA Transfection—To generate virus stocks or examine viral protein expression, 293 cells grown in 100-mm Petri dishes were
Fig. 1. Construction of HIV-1 LLP-1 point deletion and substitution mutants. The amino acid sequence in single-letter codes from residues 816-856 located in the C terminus of the HIV-1 gp41 cytoplasmic tail of the HXB2 strain is shown at the top. Single point deletions and substitutions were individually introduced into each plate for the periods described under "Experimental Procedures." Dashes indicate that the amino acids in the mutant proviruses are identical to those of the wt provirus. Deleted amino acids are indicated by Δ.

Transfections were supplemented with Nonidet P-40 and sodium deoxycholate each at a final concentration of 1%. Cells were washed twice with cold PBS and lysed with 1 ml of PBS containing 1% Nonidet P-40, 1% sodium deoxycholate, and the complete protease inhibitor mixture. After standing at 4 °C for 10 min, the cell lysates were cleared by centrifugation at 10,000 × g at 4 °C for 10 min. Equal volumes of cell lysates and culture supernatants were precipitated with 5 μl of mAb 902 acetic fluid, 5 μl of sheep anti-gp120, or 10 μl of anti-HIV-1 antisera pooled from HIV-1-infected patients. These antibodies were prebound to a mixture containing 30 μl each of protein A- and protein G-Sepharose 4B (Amersham Biosciences). After incubation at 4 °C for 2–3 h, the immune complexes were washed six times with RIPA buffer (10 mM Tris-HCl (pH 7.2), containing 1% Triton X-100, 0.1% SDS, 1% sodium deoxycholate, 5 mM EDTA, and 0.15 mM NaCl) and then boiled in 50 μl of Laemmli buffer. The proteins released from the beads were resolved by SDS-7.5% PAGE followed by fluorography.

In Vitro Transcription/Translation—In vitro wt and mutant Env synthesis was performed using the T7 polymerase-based reticulocyte TNT quick-coupled transcription/translation kit (Promega). Half a microgram each of the wt and mutant pCDNA3/env plasmids was used in a total volume of 25 μl of reaction mixture in the absence of canine pancreas microsomal membrane as described previously (41). Reactions were performed in a radiolabeled format using [35S]methionine or a non-radiolabeled format using Transcend biotinylated lysyl-tRNA according to the protocols provided by the manufacturer. Equal volumes of the [35S]-labeled or biotinylated wt and mutant reaction mixtures were directly analyzed by SDS-PAGE or subjected to immunoprecipitation with mAb 902 (for radiolabeled proteins) or Western blotting using various mAb mixtures (for biotinylated proteins).

RESULTS

Deletion of Val-833 in the gp41 LLP-1 Motif Impairs Virus Replication—To delineate the role of the LLP-1 sequence in the virus life cycle, single point deletions were individually introduced at residues Val-833, Ile-843, Ile-847, and Ile-854 in the Env protein of the HXB2 strain (Fig. 1A). Among the residues deleted, two Val residues are located adjacent to each other at positions 832 and 833 of the Env protein. The resultant Val deletion mutant is referred to as the Δ833 mutant for simplicity. These mutants were constructed using HXB2R3 as the backbone provirus by overlap PCR mutagenesis. As a control for the effects of deletions in the LLP-1 sequence, Thr-818, which is located outside the LLP-1 sequence, was also deleted (Fig. 1A). These deleted residues were located in neither the tat nor the rev open reading frames.

To examine whether these single deletions exert an effect on virus infectivity, cell-free viruses produced from 293 cells transfected with the wt provirus or with each of the mutant proviruses and normalized by RT activity were used to challenge CEM-SS cells. The wt and all mutant viruses except the
Δ833 mutant virus showed productive replication with a peak of RT production 11–15 days after infection (Fig. 2A). The Δ833 mutant did not replicate at all in CEM-SS cells even 30 days after challenge (Fig. 2A). When virus infectivity was assayed in PM1 cells, mutants Δ843, Δ847, and Δ854 all exhibited viral replication kinetics similar to that of the wt virus, although the Δ818 mutant showed replication kinetics slightly slower than that of the wt virus (Fig. 2B). The slightly reduced viral replication in the Δ818 mutant was much less significant compared with the inability of the Δ833 mutant virus to establish an infection in PM1 cells even 30 days after challenge (Fig. 2B).

The Δ833 Mutant Virus Exhibits a Reduced Steady-state Env Expression Phenotype—To delineate the nature of the defect of the Δ833 mutant virus, the expression of viral proteins was assessed by transfection of 293 cells with wt or mutant proviruses. Equal volumes of lysates obtained from transfected cells were separated by SDS-10% PAGE and analyzed by Western blotting using mAbs 902, Chessie 13, Chessie 8, and 183. Hybridoma 902 secretes a mouse mAb specific for the gp120 V3 region of the LAV and IIIB strains of HIV-1. Chessie 13 and Chessie 8 mAbs recognize residues 252–273 and 727–732, respectively, of the Env protein of the HIV-1 LAI strain. Chessie 13, Chessie 8, and 183 recognized residues 252–273, 727–732, and 732–734, respectively, of the Env protein of the HIV-1 LAI strain. Chessie 13 and Chessie 8 mAbs recognize residues 252–273 and 727–732, respectively, of the Env protein of the HIV-1 LAI strain. Chessie 13, Chessie 8, and 183 were also observed (Fig. 3A). However, gp120 was found to be associated with the wt and all mutant virions but not the Δ833 mutant virion (Fig. 3A, top panel, lanes 9–14). To better separate gp160 and gp120, additional aliquots of cell and virion lysates were resolved by SDS-7.5% PAGE followed by Western blotting using mAbs 902 and Chessie 13 (bottom panel). The migration positions of molecular mass markers in kDa are also shown. Additional aliquots of cell and virion lysates were resolved by SDS-7.5% PAGE followed by Western blotting using mAbs 902 and Chessie 13 (bottom panel). B, expression in HeLa cells. HeLa cells were transfected with 10 μg each of the wt or mutant proviruses by the LipofectAMINE transfection method. Cell and virion lysates were then resolved by SDS-10% PAGE followed by Western blotting using mAbs 902, Chessie 13, Chessie 8, and 183.

To determine whether the reduced Env steady-state expression observed in the Δ833 mutant virus was cell type-specific, viral protein expression in HeLa cells was examined. The Δ833 mutant provirus produced lower levels of cell-associated gp160 and gp120 compared with the wt and other mutant proviruses.
upon transfection, even though the production of Gag products in this mutant was normal (Fig. 3B, left panel). Again, no detectable amount of gp120 was found to be associated with the Δ833 mutant virion, although the amounts of p24, p41, and uncleaved Pr55 associated with this mutant virion were comparable with those detected in the wt and other mutant virions (Fig. 3B, right panel). The ratio of intracellular gp160 to gp120 observed in the Δ833 mutant was similar to that observed in the wt and other mutants (Fig. 3B, left panel), indicating that the Δ833 mutant Env precursor is normally processed into gp120 and mutant gp41.

We next determined whether the Δ833 mutant also exhibits a reduced steady-state Env expression pattern in human CD4+ T cells, which are natural host cells for HIV-1 infection. Because T cells are usually transfected less efficiently than required for biochemical analyses of viral protein expression and Env incorporation, a high level, transient HIV-1 expression system based on pseudotyping with VSV G protein (19, 49) was employed. For this, 293 cells were cotransfected with pHCMV-VSV G along with the wt provirus and each of the mutant proviruses. Cell-free VSV G-pseudotyped virus stocks were harvested, normalized by RT activity, and used to challenge CEM-SS. Equal portions of cell and virion lysates were analyzed by Western blotting using the mAb mixture containing the Env- and CA-specific mAbs. Infection with the wt and all mutant pseudotypes showed comparable steady-state levels of cell-associated Gag products (Fig. 4A, lanes 1–6). Similar levels of p24 were associated with the wt and all mutant virions (Fig. 4A, lanes 7–12). Again, the Δ833 mutant showed significantly lower levels of cell-associated gp160 precursor and gp120 than did the wt and other mutants (Fig. 4A, lanes 1–6), and gp120 and gp41 could not be detected in this mutant virion (Fig. 4A, lane 9). When the same samples were analyzed by Western blotting using sheep anti-gp120 antisera, similar patterns of Env steady-state expression and incorporation into virions to those observed in Fig. 4A were obtained for the wt and mutant viruses (Fig. 4B).

Expression of the Δ833 Mutant Env from an Env Subgenomic Expression Vector—To determine whether the reduced Env steady-state expression phenotype observed in the Δ833 mutant is an intrinsic characteristic of the mutant Env and is independent of other viral protein expression, the env genes in all of the mutant proviruses were subcloned into the pSV7-puro env expression vector. HeLa cells were cotransfected with pIIIex along with either the wt plasmid or each of the mutant pSV7-puro plasmids. Again, the Δ833 mutant produced lower levels of intracellular gp160, gp120, and gp41 than did the wt and other mutants (Fig. 4C).

Characterization of Viruses Encoding Point Deletions in the N Terminus of the LLP-1 Region—To determine further whether the N-terminal, but not the C-terminal, segment of the LLP-1 sequence is critical for Env steady-state expression, additional point deletions were introduced into residues at Ile-830, Ala-836, and Ile-840 (Fig. 1B). The Δ830 mutant virus, like the Δ833 mutant virus, did not replicate at all in CEM-SS cells even 35 days after virus challenge (Fig. 5A). The Δ840 mutant virus replicated with delayed kinetics compared with the wt virus with a peak of RT production between days 25 and 28. This was in contrast to the peak of RT production on day 11 observed for the wt virus (Fig. 5A). Although the Δ836 mutant virus replicated with kinetics slower than that of the wt virus, the delay in replication with this mutant was less significant than those with the Δ830 and Δ840 mutant viruses (Fig. 5A).

Viral proteins expressed by these mutants were then examined in CEM-SS cells infected with the wt or mutant VSV G pseudotypes. Deletions of residues 830, 836, and 840 did not affect the synthesis and processing of intracellular Pr55, nor did they affect virus assembly/budding (Fig. 5B). The Δ836 mutant produced comparable amounts of cell-associated gp160, gp120, and gp41 to those produced by the wt virus (Fig. 5B, compare lanes 1 with lane 1). Both the Δ830 and Δ840 mutants produced smaller amounts of intracellular Env proteins than did the wt virus (Fig. 5B, compare lanes 3 and 5 with lane 1), with the Δ830 mutant showing a greater reducing effect on Env steady-state expression than did the Δ840 mutant. However, the Δ830 mutant produced slightly larger amounts of cell-associated Env proteins than did the Δ833 mutant (Fig. 5B and data not shown). Comparable amounts of gp120 and gp41 to

**Fig. 4. Synthesis of viral proteins encoded by deletion mutants.** A and B, assessment of viral protein expression by VSV G-pseudotype infection. 293 cells were cotransfected with 7.5 μg of pHCMV-VSV G and 7.5 μg each of the wt or mutant proviruses. Cell-free VSV G-pseudotyped recombinant viruses containing 7 × 10⁵ cpm of RT activity obtained from each transfection were used to challenge 2 × 10⁶ CEM-SS cells. After overnight incubation, cells were washed with PBS and allowed to incubate at 37 °C for an additional 2 days. Equal portions of cell and virion lysates were analyzed by SDS-10% PAGE followed by Western blotting using mAbs 902, Chessie 13, Chessie 8, and 183 (A). The same samples were also analyzed by Western blotting using sheep anti-gp120 (B). C, analysis of mutant Env proteins encoded by the pSV7-puro expression vector. HeLa cells were cotransfected with pHIexat together with the wt or mutant pSV7-puro plasmids. Cotransfection with pHIexat and pSV7(ΔKS)-puro (40), a derivative of pSV7-puro in which the Kpol site (at nucleotide position 6351 of the HXB2 sequence) to the Sfil site (positioned at 6834) is deleted, was used as a negative control. Two days after transfection, lysates obtained from each transfection were resolved by SDS-7.5% PAGE followed by Western blotting using mAbs 902, Chessie 13, and Chessie 8.
wheras no gp120 or gp41 could be detected in the viral protein expression in CEM-SS cells. VSV-G-pseudotyped virus production measured by RT activity was monitored after infection. 

Because deletion of one of the Env Steady-state Expression—

Effects of Amino Acid Substitutions at Val-832 and Val-833 on Env Steady-state Expression—Because deletion of one of the adjacent Val residues located at positions 832 and 833 displayed the most significantly reduced Env steady-state expression phenotype among the deletion mutants analyzed, we next determined the amino acid specificity of Val-832 and Val-833 on Env steady-state expression. Mutant viruses encoding Env in which Val-832 and Val-833 were each replaced by the nonconserved residues Ala, Ser, and Pro were examined (Fig. 1C). These substitutions were expected to alter the α-helical structure in the increasingly severe order of Pro > Ser > Ala (50). The 832A and 832S mutants showed wt virus-like replication kinetics, whereas the 832P mutant exhibited slightly delayed replication kinetics compared with that of the wt virus (Fig. 6A). The 833A and 833S mutants also displayed wt virus-like replication kinetics (Fig. 6B). Like the Δ833 mutant, the 833P mutant did not replicate at all even 31 days after challenge to CEM-SS cells (Fig. 6B).

VSV G-pseudotype infection of CEM-SS cells was employed to assess viral protein expression in these substitution mutants. All of the Val-832 and Val-833 substitution mutants produced similar amounts of intracellular and virion-associated gag gene products to those produced by the wt virus (Fig. 6C). Among these mutants, only the 833P mutant showed significantly reduced levels of intracellular gp160, gp120, and gp41 compared with the wt and other mutants (Fig. 6C, lanes 1–8). In addition, gp120 and gp41 could not be detected in this mutant virion (Fig. 6C, lane 16).

The Δ833 Mutant Env Rapidly Degrades After Biosynthesis—We then explored the mechanism responsible for the reduced Env steady-state expression of the Δ833 mutant. First, cells cotransfected with the wt or Δ833 mutant pSVE7-puro plasmid along with pIIExtat were labeled with [35S]methionine at 37 °C for 10 min. Cell lysates containing the wt or Δ833 mutant Env were individually precipitated with sheep anti-gp120, pooled human anti-HIV-1, and mAb 902. A similar or even a slightly greater amount of the Δ833 mutant gp160 precursor than that of the wt precursor was precipitated by mAb 902 (Fig. 7A). These observations indicated that mAb 902, like polyclonal anti-gp120 and anti-HIV-1, reacts with the Δ833 mutant gp160 precursor as effectively as with the wt gp160. Because of the availability of hybridoma 902 and the lower background precipitated by this mAb compared with other polyclonal antibodies, mAb 902 was used in later studies. Next, HeLa cells expressing the wt or Δ833 mutant Env were metabolically labeled at 37 °C for different times, and cell lysates were immunoprecipitated with mAb 902. Similar amounts of gp160 precursor were detected in both the wt and Δ833 mutant for each labeling time (Fig. 7B), indicating that deletion of Val-833 does not affect de novo Env biosynthesis.

To determine whether the reduced steady-state Env expression of the Δ833 mutant is due to the rapid turnover of this mutant after synthesis, a pulse-chase experiment was performed. The levels of the intracellular Δ833 mutant Env precursor declined more rapidly than those of the wt Env as the chase proceeded (Fig. 7C). The percentage of radioactivity of each species at each indicated chase time relative to the radioactivity of the gp160 precursor at chase time 0 was quantitated by an instant imager and then calculated. The Δ833 mutant Env precursor displayed a more rapid turnover rate than the wt precursor (Fig. 7D). However, the faster decrease in intracellular Δ833 mutant gp160 compared with that of intracellular wt gp160 was not concomitant with the more rapid appearance of intracellular or extracellular gp120 in this mutant (Fig. 7D, right panel). This observation indicates that the greatly reduced Env steady-state expression observed in the Δ833 mutant is not due to the enhanced dissociation of gp120 from the gp120-mutant gp41 complex nor is it due to the subsequent rapid secretion of gp120 into the culture medium.

Rapid Degradation of Δ830 and 833P Mutant Env Proteins in Cells After Synthesis—Because the two mutants Δ830 and 833P also exhibited reduced Env steady-state expression compared with the wt virus, the efficiency of de novo Env synthesis in these two mutants was compared with that of the wt Env in a study utilizing 10-min pulse labeling. Similar amounts of the wt, Δ830, and 833P gp160 precursors were precipitated by either anti-HIV-1 or mAb 902 (Fig. 8A). When a pulse-chase experiment was performed, the Δ830 and 833P mutant Env proteins, like that of the Δ833 mutant Env, showed faster turnover kinetics than did the wt Env (Fig. 8, B and C).

Different mAb Mixtures Equivalently Recognize the wt and Mutant Env Proteins—To address directly the issue of whether mAb 902 precipitates the wt, Δ830, Δ833, and 833P Env proteins equivalently in labeling and pulse-chase studies, in vitro coupled transcription/translation in the absence of microsomal membranes was performed in a radiolabeled format using

**A**, infectivity assay of Val-832 substitution mutants. 293 cells were transfected with the wt or 832 mutant proviruses as indicated. Cell-free viruses containing $2 \times 10^4$ cpm of RT activity obtained from each transfection were used to challenge CEM-SS cells, and RT activity was monitored postinfection.

**B**, infectivity assay of Val-833 substitution mutants. 293 cells were transfected with the wt or 833 mutant proviruses, and virus infectivity was assayed as described in **A**.

**C**, expression of viral proteins encoded by point substitution mutants. Cell-free, VSV G-pseudotyped recombinant viruses as indicated containing $10^6$ cpm of RT activity were used to challenge CEM-SS cells. Equal portions of cell lysates and virion fractions were then analyzed by Western blotting using mAbs 902, Chessie 13, Chessie 8, and 183.
pCDNA3/env plasmids that encode the full-length wt or Δ830, Δ833, and 833P mutant env genes downstream of a T7 promoter. Comparable levels of trichloroacetic acid-precipitable counts were obtained in equal volumes of the wt and mutant reaction mixtures (data not shown). When equal volumes of the [35S]methionine-labeled wt and mutant reaction mixtures were

FIG. 7. Assessment of the synthesis and degradation of Δ833 mutant Env. A, immunoprecipitation of Env proteins by various antibodies. Two days after transfection, HeLa cells expressing wt or Δ833 mutant Env were labeled with [35S]methionine at 37 °C for 10 min. Equal volumes of cell lysates were precipitated with sheep anti-gp120, pooled human anti-HIV-1 antisera, or mAb 902 as indicated, and precipitated proteins were analyzed by SDS-7.5% PAGE followed by fluorography. B, analysis of Env synthesis. HeLa cells expressing wt or Δ833 mutant Env were labeled with [35S]methionine at 37 °C for different times, and equal volumes of cell lysates were immunoprecipitated with mAb 902 followed by SDS-7.5% PAGE. C, pulse chasing of Env proteins. HeLa cells expressing wt or Δ833 mutant Env were metabolically labeled at 37 °C for 30 min and then chased for different times in the presence of excess cold methionine. Equal volumes of cell lysates and extracellular fractions obtained from each chase time were precipitated with mAb 902 followed by SDS-PAGE. D, kinetics of processing and extracellular secretion of [35S]-labeled wt and mutant Env proteins. The gels shown in C were scanned with an Instant Imager (Packard Instrument Co.). The bands corresponded to intracellular gp160 and gp120, and extracellular gp120 of the wt and Δ833 mutant were quantitated. The radioactivity of each gp120 band was calibrated by multiplying by a factor of 4/3 because the numbers of methionine residues present in gp120 and gp41 are 12 and 4, respectively. In each gel, the relative percentage of the radioactivity of each Env species for each chase time to the radioactivity of intracellular gp160 at chase time 0 was calculated and plotted versus the chase time.
directly subjected to SDS-PAGE, similar amounts of the wt and mutant proteins, which all migrated as a predominant band with an apparent molecular mass of 90 kDa, were detected (Fig. 9A). This molecular mass was consistent with the molecular weight of the unglycosylated form of Env. These observations also indicate that highly homogenous wt and mutant proteins are comparably synthesized under this in vitro protein synthesis condition. Next, equal volumes of the wt and mutant reaction mixtures were immunoprecipitated with pooled anti-HIV or mAb 902. mAb 902, as well as anti-HIV-1, reacted equivalently with the wt, Δ830, Δ833, and 833P mutant proteins synthesized in vitro (Fig. 9B).

To address whether the mAbs mixtures used in Western blot analyses recognize the wt and mutant proteins equivalently, in vitro protein synthesis was performed in a non-radiolabeled format using Transcend biotinylated lysyl-tRNA. Equal volumes of the wt and mutant reaction mixtures were analyzed by Western blotting using streptavidin-conjugated HRP to detect total proteins synthesized in vitro. Similar amounts of the wt and mutant proteins were detected (Fig. 9C). Equal volumes of the wt and mutant reaction mixtures were then analyzed by Western blotting using various combinations of mAbs. After the first antibody incubation, blots were incubated with HRP-conjugated second antibody directed against mouse IgG. All mAb mixtures, i.e. 902 + Chessie 13 + Chessie 8 + 183, 902 + Chessie 13 + Chessie 8, and 902 + Chessie 13, reacted with the wt protein and each of the mutant proteins synthesized in vitro equivalently (Fig. 9D).

Rev Was Comparably Synthesized in the wt and Mutant pSVE7-puro Transfections—To rule out the possibility that the decreased levels of the Δ830, Δ833, and 833P mutant precursors are due to reduced Rev synthesis by these three mutant pSVE7-puro transfections in cells, equal volumes of cell lysates from each transfection, which contained comparable amounts of β-actin (Fig. 9E, bottom panel), were analyzed by Western blotting using an HIV-1 Rev mAb. Similar amounts of the Rev protein, which migrated as a 16.5-kDa species, were detected in the wt transfection and all mutant pSVE7-puro transfections (Fig. 9E, top panel).

DISCUSSION

Although previous truncation and deletion analyses have implicated involvement of the cytoplasmic tail, or possibly the LLP-1 region, of gp41 in Env stability, the precise effects of such large truncations or deletions on Env stability are not fully understood. Large truncations or deletions in a protein
inevitably result in drastic structural and folding alterations and/or disruption in interactions with cellular factors. The observed phenotypic changes of these deletion Env mutants cannot be simply attributed to a loss of functions that results from the lack of target sequences in Env. In our initial study to address the importance of amino acid residues located in the LLP-1 region in virus infection, we examined HIV-1 mutant viruses that encoded point deletions at Thr-818, Val-833, Ile-843, Ile-847, and Ile-854 in Env of the HXB2 strain. Only the \( H9004_{833} \) mutant greatly reduced Env steady-state expression, which, in turn, resulted in impaired virus infectivity and gp120 incorporation into this mutant virion (Fig. 2, Fig. 3, and Fig. 4, A and B). The \( H9004_{833} \) mutant also showed significantly reduced steady-state Env expression in the absence of other viral proteins (Fig. 4C), indicating that Env expressed from the pSVE7-puro plasmid represents that in the context of replication of the entire HIV-1 genome. The reduced Env steady-state expression profile of the \( H9004_{833} \) mutant was observed in various cell types including 293, CEM-SS, and HeLa cells (Fig. 3 and Fig. 4), implying that this feature of the \( H9004_{833} \) mutant Env is not a cell type-specific phenomenon.

To define further the involvement of the N terminus of the LLP-1 motif in Env stability, we examined the \( H9004_{830}, \ H9004_{836}, \) and \( H9004_{840} \) mutants. The \( H9004_{840} \) mutant reduced

---

**Fig. 9. Characterization of Env proteins synthesized in vitro and assessment of Rev synthesis in pSVE7-puro transfections.** A and B, analysis of \(^{35}\)S-methionine-labeled wt and mutant proteins. pCDNA3/env plasmid encoding wt or mutant proteins as indicated were used in in vitro coupled transcription/translation reactions in the absence of microsomal membranes to produce \(^{35}\)S-methionine-labeled proteins. After synthesis, excess cold methionine was added to the mixtures, and the reaction mixtures were allowed to incubate at 30 °C for an additional 30 min. Equal volumes (2 \( \mu \text{l} \)) of the wt and mutant reaction mixtures were directly separated by SDS-7.5% PAGE (A). Another aliquot (7 \( \mu \text{l} \)) of each reaction mixture was first immunoprecipitated with pooled anti-HIV-1 antisera or mAb 902, and the precipitated proteins were then resolved by SDS-7.5% PAGE (B). C and D, analyses of biotinylated wt and mutant proteins. Equal volumes (5 \( \mu \text{l} \)) of biotinylated wt and mutant in vitro reaction mixtures were directly separated by SDS-7.5% PAGE followed by Western blotting using streptavidin-conjugated HRP to detect total in vitro synthesized proteins (C). After stripping off streptavidin-linked HRP from the immune complexes, the blot shown in C was reprobed with mAbs 902 + Chessie 13 + Chessie 8 and 183 (D, top panel). Other aliquots (5 \( \mu \text{l} \)) of the wt and mutant reaction mixtures were analyzed by Western blotting using mAbs 902 + Chessie 13 + Chessie 8 (D, middle panel). After stripping off antibodies from the immune complexes, the blot shown in the middle panel of D was reprobed with mAbs 902 + Chessie 13 (D, bottom panel). In each case, HRP-conjugated antibody against mouse IgG was used as a second antibody to detect the nonglycosylated wt and mutant proteins. E, analysis of Rev synthesis. Equal volumes of lysates from HeLa cells expressing wt or mutant Env proteins were subjected to SDS-15% PAGE followed by Western blot analysis using an HIV-1 Rev mAb. The same cell lysates were also resolved on a 10% gel followed by Western blotting using a \( \beta \)-actin mAb.
steady-state Env expression compared with the wt virus, but the reduction in the Env steady-state level in this mutant was less prominent than that observed in the Δ830 mutant (Fig. 5B). Parallel to these observations, gp120 and gp41 could not be detected in the Δ830 mutant virion and were barely detectable in the Δ840 mutant virion (Fig. 5B). The reduced Env steady-state expression thus delays or impairs the viral infectivity of these two mutants (Fig. 5A). Although deletion of Ala-836 did not greatly affect the intracellular levels of Env proteins nor alter gp120 and gp41 incorporation into the mutant virion (Fig. 5B), this mutant replicated with kinetics slightly slower than that of the wt virus (Fig. 5A). Because the cytoplasmic tail is not essential for Env-mediated syncytia formation (4, 5, 51), it appears that deletion of Ala-836 may marginally affect some step that occurs after receptor binding and membrane fusion.

To delineate which Val residue located adjacent to positions 832 and 833 is critical for steady-state Env expression, mutants with Val-832 and Val-833 individually replaced by the nonconserved amino acids Ala, Ser, and Pro were examined. Among all substitution mutants examined, only a Val-to-Pro substitution at residue 833 profoundly reduced Env steady-state expression, which is concomitant with severely impaired Env incorporation into virions and infectivity of the mutant virus (Fig. 6). The effect on Env steady-state expression of Pro substitution at Val-833 is specific because Pro substitution for Val-832 did not alter Env steady-state expression (Fig. 6C).

The possibility that mutations in the N terminus of the LLP-1 motif induce a conformational change that hinders recognition of the mutant Env proteins by Env-specific mAbs used can be eliminated based on the following studies. First, to address the issue of whether the mAb mixtures used recognized the wt and mutant proteins equivalently, in vitro transcription/translation assays were performed in the absence of microsomal membranes. This in vitro coupled transcription/translation provides a ready protein synthesis system to express sufficient amounts of [35S]methionine or biotinylated wt and mutant proteins with a high degree of purity for biochemical analyses without further purification. Unlike mutant Env synthesis in cells, in vitro production of Env proteins in the absence of microsomal membranes results in non-glycosylated Env species that do not undergo precursor processing and degradation. Also, the amounts of in vitro synthesized Env proteins can be directly assessed by detection of the radiolabeled or biotinylated proteins in a given volume. In fact, equal volumes of the wt and mutant reaction mixtures contained comparable amounts of the [35S]-labeled or biotinylated wt and mutant proteins (Fig. 9, A and C), indicating that the wt and mutant proteins are comparably synthesized in vitro. In addition, these in vitro synthesized proteins were highly homogenous, as judged by the detection of a predominant band of the wt and mutant proteins when the reaction mixtures were directly resolved by SDS-PAGE (Fig. 9, A and C). mAb 902 precipitated equivalently the wt, Δ830, Δ833, and 833P mutant proteins synthesized in vitro (Fig. 9B). Also, all mAb mixtures used in the study, i.e. 902 + Chessie 13 + Chessie 8 + 183, 902 + Chessie 13 + Chessie 8, and 902 + Chessie 13, equivalently recognized the in vitro synthesized wt, Δ830, Δ833, and 833P mutant proteins (Fig. 9D). We observed that the amounts of Δ830, Δ833, and 833P mutant gp160 precursors precipitated by mAb 902 were comparable with the amount of wt gp160 precipitated when cells expressing these mutant proteins were labeled for a short period, ranging from 5 to 30 min (Fig. 7, A and B, and Fig. 8A). This is also consistent with the notion that mAb 902 precipitates the wt and mutant proteins equivalently.

Env expression from the pSVE7-puro plasmid is Rev-dependent. Because all of the mutations examined in the present study are not located in the tat or rev open reading frame, the rev-coding sequence in all mutant pSVE7-puro plasmids should remain intact, and Rev should presumably be expressed comparably by the wt and all mutant plasmids. Because Gag expression in HIV-1-infected cells is also Rev-dependent, the observations that all of the mutants examined in this study produced comparable amounts of Gag proteins to those produced by the wt virus (Figs. 3–6) indicate that mutations in the LLP-1 motif do not affect Rev expression. This notion is directly proven by the observation that comparable amounts of Rev were detected in cells transfected with the wt or mutant pSVE7-puro along with pHIVex (Fig. 9E). Therefore, the time-dependent decrease in intracellular gp160 levels observed in these mutants at later chase periods, but not during pulse labeling (Figs. 7 and 8), reveals that the mutant Env proteins are unstable after biosynthesis, presumably due to their time-dependent transport to an intracellular site where they degrade rapidly.

The conserved residues located in the LLP-1 region are not necessarily crucial for Env stability. Ile-830, Ile-843, and Ile-847 are highly conserved among different HIV-1 clades (Fig.
A deletion in an α-helix is expected to skew the structure in a way that residues originally aligned on one face of the helix become displaced. The differential effect of deletions in the N-terminal and C-terminal regions of LLP-1 on Env stability suggests that the structure defined by the N terminus of the LLP-1 motif is critical for maintaining Env stability, but that the functions of Env can tolerate alterations to the C-terminal region of the LLP-1 sequence. Moreover, substitutions by the nonconserved amino acids Ala, Ser, and Pro at Val-833 are expected to disrupt the local α-helical structure of the N terminus of the LLP-1 motif in an increasingly severe manner. This hypothesis is supported by previous peptide modeling studies on the leucine zipper-like motif of HIV-1 gp41. Substitution of Ile-573 by a Ser residue in a peptide mimicking this domain more greatly decreases the stability of the coiled-coil structure of the peptide than that which occurs by an Ala residue, whereas a peptide containing an Ile-to-Pro substitution exhibits no stable α-helical structure in solution (50). There appears to be a correlation between the degree of destabilization of Env by Ala, Ser, and Pro substitutions for Val-833 and the ability of these substitutions to alter the α-helical structure of the N terminus of the LLP-1 region (Fig. 6C and Fig. 8C). These studies further substantiate the proposal that the C-terminal domain in gp41 during virus infection.

The mechanism underlying the rapid degradation of Env proteins with mutations in the N terminus of the LLP-1 motif is not yet understood. It is likely that the local structural perturbation in the N terminus of the LLP-1 sequence induced by Ile-830 or Val-833 deletion or a Val-to-Pro substitution at residue 833 may alter the overall folding of Env, thus causing these mutant proteins to be routed to an intracellular compartment where the mutant Env proteins rapidly degrade. There is a precedent for this hypothesis. Truncation of the cytoplasmic tail of the simian immunodeficiency virus Env results in an alteration in the conformation of the ectodomain of the TM protein on the cell surface (53). Alternatively, the N terminus of the LLP-1 motif may be directly involved in interactions with cellular factors that modulate Env folding, transport, and/or stability. Deletions of these residues thus alter Env trafficking and/or stability.

Previous studies (5, 9, 10, 12, 13, 51, 54) showed that deletions or truncations of regions encompassing residues 830–833 do not apparently affect Env stability. Hunter and co-workers (5, 8) also examined mutants with truncations from the C terminus of the cytoplasmic tail or with deletions of multiple consecutive residues or multiple substitutions at different sites located in the conserved C terminus of the gp41 cytoplasmic tail. Except for the C-terminal 19-amino acid deletion mutant that is unstable and rapidly degrades after biosynthesis, all other deletion mutants do not seem to affect Env stability, even though these mutations may reduce virus infectivity and Env incorporation into virions. Even the longest deletion mutant Δ351, which lacks the sequence between Ile-820 and Ala-839, still replicates in CD4+ T cells but with delayed kinetics (8). In the present study, we demonstrate that Ile-830 and Val-833 located in the N terminus of the gp41 LLP-1 motif have a critical role in modulating HIV-1 Env stability. The Ile-830 and Val-833 mutations likely induce a change in Env conformation that targets mutant Env by a cellular degradation pathway. This study has implications toward understanding how HIV-1 Env expression is modulated by the structure of an intracytoplasmic domain in gp41 during virus infection.

Acknowledgments—The pHCMV-VSV G plasmid was a kind gift from J. C. Burns (University of California School of Medicine, San Diego). We acknowledge the help of the National Institutes of Health AIDS Research and Reference Reagent Program in providing the reagents listed below, especially P. L. Naras (CEM-SS), M. Reitz, Jr. (PMI), B. Chesebro (hybridoma 902 and 183), G. K. Lewis (hybridoma Cheesie 13 and Cheesie 8), and M. Phelan (sheep anti-gp120). The HIV-1 Env-Ab, donated by J. P. Compans (Washington, DC), was provided by the EU Program EVA/MRC Centralized Facility for AIDS Reagents, National Institute for Biological Standards and Control, UK (supported by Grant QLK2-CT-1999-00609 and GP282102).
N Terminus of the LLP-1 Motif and HIV-1 Env Stability

Effect of Point Mutations in the N Terminus of the Lentivirus Lytic Peptide-1 Sequence of Human Immunodeficiency Virus Type 1 Transmembrane Protein gp41 on Env Stability

Sheau-Fen Lee, Chiung-Yuan Ko, Chin-Tien Wang and Steve S.-L. Chen

doi: 10.1074/jbc.M201479200 originally published online February 21, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M201479200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 37 of which can be accessed free at http://www.jbc.org/content/277/18/15363.full.html#ref-list-1