The E1 and E2 glycoproteins of hepatitis C virus form a noncovalently associated heterodimer that mediates viral entry. Glycoprotein E2 comprises a receptor-binding domain (residues 384–661) that is connected to the transmembrane domain (residues 716–746) via a highly conserved sequence containing a hydrophobic heptad repeat (residues 675–699). Alanine- and proline-scanning mutagenesis of the E2 heptad repeat revealed that Leu³⁷³, Ser³⁷⁶, Leu³⁷⁸, and Leu⁶⁶⁵ are important for E1E2 heterodimerization. Furthermore, Pro and Ala substitution of all buried heptad repeat residue (Ser³⁷³) blocks the entry of E1E2-HIV-1 pseudotypes into Huh7 cells, irrespective of an effect on heterodimerization. Two conserved prolines (Pro⁶⁷⁶ and Pro⁶⁸³), occupying consecutive b positions of the heptad, were not required for E1E2 heterodimerization; however, Pro⁶⁸³ was critical for viral entry. Thus, disruption of the predicted a-helical structure by proline at position 683 is important for E2 function. The inability of mutants to mediate viral entry was not explained by a loss of receptor binding function, because all mutants were able to interact with a recombinant form of the CD81 large extracellular loop. Chimeras formed between the E1 and E2 ectodomains and the transmembrane domains of flavivirus prM and E glycoproteins, respectively, were able to heterodimerize, although with lower efficiency in comparison with wild type E1E2. The heptad repeat of E2 therefore requires the native transmembrane domain for full heterodimerization and viral entry function. Our data indicate that the membrane-proximal heptad repeat of E2 is functionally homologous to the stem of flavivirus E glycoproteins. We propose that E2 has mechanistic features in common with class II fusion proteins.

Hepatitis C virus (HCV) chronically infects 3% of the world population and is the leading indicator of liver transplantation in Western countries. Currently, no vaccine exists to prevent infection, and therapeutic agents such as pegylated interferon and ribavirin have limited success in achieving viral clearance. A more complete understanding of the viral life cycle, including viral entry, is essential so that new targets for viral inhibition are found.

HCV is a member of the Flaviviridae family of viruses and encodes two viral envelope glycoproteins, E1 and E2. E1 and E2 are cleaved cotranslationally from a polyprotein precursor, forming covalently and noncovalently associated heterodimers in the endoplasmic reticulum. A small proportion of noncovalently associated heterodimers enter the secretory pathway to become surface expressed (1). The surface-expressed E1E2 heterodimer represents a functional glycoprotein form because it can mediate entry of E1E2-retrovirus pseudotypes into hepatic cells (1–3). Inhibitors of vacuolar acidification block the entry of E1E2-retrovirus pseudotypes into Huh7 cells (3), indicating that E1E2-mediated entry occurs via receptor-mediated endocytosis, with the low pH environment of the endosome triggering membrane fusion. E2 has an essential role in receptor binding (4–7); however, its role in membrane fusion remains uncharacterized. The functional role of E1 is unknown; however, its association with E2 is essential for viral entry (1–3).

Functional paradigms for HCV E1 and E2 may be found with the phylogenetically related flavivirus glycoproteins, prM and E, which also form noncovalent heterodimers (8). Glycoprotein E has the dual functions of receptor binding and membrane fusion (9, 10), whereas prM appears to protect E from premature fusion activation during passage through the secretory pathway (8). A soluble 385-amino acid fragment of E, containing the receptor-binding domain and fusion peptide, can be cleaved by trypsin from purified flavivirus (e.g. tick-borne encephalitis virus) (10). X-ray crystallography has revealed that this soluble E fragment is composed of three subdomains (I, II, and III) that are rich in b-sheets, typical of class II fusion proteins (10). At neutral pH, E monomers are arranged as head-to-tail dimers, lying parallel to the viral membrane (12). Exposure to low pH causes a reorientation of the three subdomains such that domain II tilts up toward the target membrane for fusion loop insertion and E dimers reassemble into trimers (13). This soluble fragment of E is connected to the transmembrane domain (TMD) via an ~50-amino acid "stem". The stem of tick-borne encephalitis virus E comprises two putative a-helices: a membrane-proximal C-terminal helix contributes to prM-E heterodimer stability at neutral pH, and an N-terminal helix promotes trimer formation at low pH (14). In the absence of the stem, E trimerization triggered by low pH is dependent on the presence of lipid (14).

An HCV E2 fragment comprised of residues 384–661 (E2(384–661)) may be homologous to the soluble ectodomain fragment of E (15). E2(384–661) mediates binding to a variety of cellular receptors, including CD81 and scavenger receptor type B class I, that play
important roles in viral entry, possibly in a coreceptor complex (16, 17). In addition, monoclonal antibodies (mAbs) specific to epitopes located within E2p61 can neutralize the infectivity of E1E2-pseudotyped particles into HuH7 cells, with low pH altering the exposure of mAb epitopes (3, 18). Like its putative flaviviral counterpart, E2p61 is linked to the TMD through a conserved sequence that is predicted to form an amphipathic α-helix containing a hydrophobic heptad repeat (Fig. 1). In this study, we have shown by proline- and alanine-scanning mutations that this heptad repeat sequence is important for E1E2 heterodimerization and for entry of E1E2-pseudotyped HIV-1 particles into HuH7 cells. We propose that the heptad repeat region of HCV E2 is a functional homolog of the flavivirus glycoprotein E stem.

EXPERIMENTAL PROCEDURES

Cell Lines and Antibodies—HEK 293T, HuH7, and Cos-7 cells were maintained in Dulbecco’s minimal essential medium containing 10% fetal calf serum and 2 mM L-glutamine (DMF10). Monoclonal antibodies H53 (19), A11, and A4 (20) were kind gifts from Drs. Jean Dubuisson and Harry Greenberg. Immunoglobulin G was purified from plasma obtained from an HIV-1-infected individual prior to SDS-PAGE and phosphorimaged in RIP lysis buffer and immunoprecipitated with mAb H53 or IgG. The chimera was pBRTM/1-3011 (a gift from Charles Rice). The HIV-1 E1CACTTAATGGCCCAGGACGCG. The HCV template used to construct the chimera was pCDNA4HisMax (Invitrogen)-based expression vector for E1, pE1E2H77c, which has been described previously (1). The E1E2-yellow fever virus chimera clone E1g/E2g was constructed using overlapping primers encoding the prM and E TMDs of yellow fever virus strain 17D. The E1 primers were forward, 5′-GGGTTACACCATGTTGGTTGCTTCTTCTATC, and reverse, 5′-GAAAAAGGTTCTCACCAGTGCTGATCCAAAGG; E2 primers were forward, 5′-GCTTGGCTTGGCTTGCGCCAGGCGAAACCAGC, and reverse, 5′-CCCACATGATCGTCTATATCTCATATTGAAAGATGAGGCGCG. The HCV template used to construct the chimeras was pBRTM1–3011 (a gift from Charles Rice). The HIV-1 luciferase reporter vector pNL4–3.LUC.R-E was obtained from Dr. N. Landau through the National Institutes of Health AIDS research and reference reagent program (21). In vitro mutagenesis was carried out by standard overlap extension PCR techniques. Inserts were fully sequenced on an ABI automated sequencer. Vectors were transfected into HEK 293T and Cos-7 cells using FuGene 6 (Roche Applied Science) as described (1).

Radioimmunoprecipitation (RIP)—Radioimmunoprecipitations were performed as described (1). HEK 293T cells transfected with plasmid DNA using FuGene 6 (Roche Applied Science) were radiolabeled with 150 μCi of Tran-35S-label (ICN, Costa Mesa, CA). Western Blot—Cell lysates prepared from transfected 293T cells were separated by SDS-PAGE and transferred to nitrocellulose membranes for Western blotting using enhanced chemiluminescence (Roche Applied Science) as described (1).

E1E2-pseudotyped HIV-1 Particle Entry Assay—Pseudotyped particle entry assays were performed as described (1). Briefly, supernatants from HEK 293T cells co-transfected with an E1E2 expression vector or cDNA4 empty vector and pNL4–3.LUC.R-E were filtered (0.45 μm) and applied to HuH7 cell monolayers. Luciferase was measured with a Microlumat LB (Berthold) luminometer or Polarstar (BMG labtechnologies) according to the manufacturer's recommendations (Sigma) and read at 450 nm.

RESULTS

Leu757, Ser678, Leu689, and Leu690 of the Membrane-Pseudotyped Virus Entry Region of E2 Are Essential for E1E2 Heterodimerization—A highly conserved hydrophobic heptad repeat (residues 675–699) is encompassed by the sequence linking E2p61 to the TMD (Fig. 1). The algorithm of Combet et al. (22) predicts that the E2 heptad repeat region will form an amphipathic α-helix on the C-terminal side of two conserved proline residues, Pro676 and Pro683, that occupy consecutive b positions. A conserved heptad repeat of similar length is also found in the membrane-proximal stem region of flaviviral E glycoproteins (Fig. 1), playing an important role in prM-E heterodimerization (14). To examine the functional role of the HCV E2 hydrophobic heptad repeat, we performed alanine- and proline-scanning mutagenesis of the a and d positions in the context of an E1E2 polymerase expression vector, pE1E2H77c (1).

We first examined how the mutations affected the synthesis and assembly of E1 and E2 into noncovalently associated heterodimers. Transfected cells were pulse-chase biosynthetically labeled, and E1E2 heterodimers were immunoprecipitated from cell lysates with the conformation-dependent anti-E2 mAb H53. Immunoprecipitations were analyzed using SDS-PAGE under non-reducing conditions. Fig. 2A, RIP, shows that reduced levels of E1 were coprecipitated with E2 when Leu757, Ser678, Leu689, and Leu690 were mutated to proline, indicating that heterodimerization is blocked by these mutations. Western blotting of the same lysates with an anti-E1 mAb (A4) revealed similar levels of E1 expression for all mutants (Fig. 2A, Western blot). These results indicate that the L675P, S678P, L689P, and L692P mutants (Fig. 2A) revealed similar levels of E1 expression for all mutants (Fig. 2A, Western blot). These results indicate that the L675P, S678P, L689P, and L692P mutations did not indirectly affect E1 expression and/or stability. In most cases, the effects of alanine substitutions were similar to those observed with proline, Serine 678 was an exception; the proline mutant lacked heterodimerization ability, whereas the Ala mutant exhibited wild type levels of E1E2 heterodimer formation (Fig. 2A).

A component of noncovalently associated E1E2 heterodimers enters the secretory pathway, becoming expressed at the cell surface (1). The surface-expressed E1E2 heterodimer repre-
Fig. 2. A, RIP, heterodimerization of E1E2 heptad repeat mutants in cell lysates. Metabolically labeled lysates of 293T cells transfected with wild type (WT), mutated pE1E2H77c, or empty cDNA4 vector (no E1E2) were immunoprecipitated with the conformation-dependent anti-E2 mAb H53. The immunoprecipitants were examined by nonreducing SDS-PAGE on 10–15% polyacrylamide gradient gels to Western blotting with the E1-specific mAb A4. B, heterodimerization of E1E2 heptad repeat mutants incorporated into HIV-1 pseudotypes. Metabolically labeled viral particles were pelleted from the tissue culture fluid prior to lysis in RIP buffer. E1E2 heterodimers were immunoprecipitated with mAb H53 prior to SDS-PAGE on 10–15% polyacrylamide gradient gels and phosphorimage analysis. C, incorporation of HIV-1 structural proteins into E1E2-pseudotyped particles. Metabolically labeled viral particles were pelleted from the tissue culture fluid prior to lysis in RIP buffer. HIV-1 proteins were immunoprecipitated with IgG from an HIV-1-infected individual prior to SDS-PAGE on 7.5–15% gradient gels and phosphorimage analysis.

Fig. 3. A, entry of wild type (WT) and proline-mutated E1E2-HIV-1 1-pseudotypes into Huh7 cells. The data represent the percentage entry of mutant versus wild type (means ± S.E.; representative of two independent experiments performed in quadruplicate). B, entry of wild type (WT) and alanine-mutated E1E2-HIV-1 pseudotypes into Huh7 cells. The data represent the percentage entry of mutant versus wild type (means ± S.E.; four independent experiments performed in quadruplicate).

sents a functional glycoprotein form because it can mediate viral entry when incorporated into retroviruses (1–3). We employed the E1E2-HIV-1 pseudotyping system to ask whether the heptad repeat mutations affect the functional E1E2 heterodimer. 293T cells were cotransfected with pE1E2H77c and pNL4.3.Luc.R-E vectors and pulse-chase biosynthetically labeled. E1E2 heterodimers were immunoprecipitated from lysed viral pellets with mAb H53 prior to SDS-PAGE. Similar levels of wild type and mutated E2 were incorporated into HIV-1 particles (Fig. 2B). As was observed for the intracellular glycoprotein complex, S678P, L689P, and L692P also blocked heterodimerization with E1 in a viral context (Fig. 2B). The L675P mutation, which exhibited diminished intracellular E1E2 heterodimerization, caused complete blockade of glycoprotein heterodimerization in a viral context. Proline at 675 is therefore not tolerated in the mature glycoprotein complex. The Ala substitutions were less severe than corresponding Pro mutations, with L675A, L689A, and L692A substitutions reducing the amount of E1 present in viral E1E2 heterodimers. Immunoprecipitation of virus lysates with IgG from an HIV-1-infected individual indicated that the HIV-1 gag structural proteins and pol replicative enzymes were incorporated at similar levels for wild type and all mutants (Fig. 2C). These data indicate that the membrane-proximal heptad repeat region of E2 is a determinant of E1E2 heterodimerization involving Leu675, Ser678, Leu689, and Leu692.

Entry of E1E2-HIV-1 Pseudotypes into Huh7 Cells—E1E2-pseudotyped retroviral particles are competent to enter hepatic cells via the endosomal pathway. Viral entry requires both E1 and E2 and can be blocked by mAbs to E2, by serum or plasma from HCV-infected individuals, and by soluble CD81 receptor (2, 3). We used the E1E2-HIV-1 luciferase reporter pseudotyping system to determine whether the heptad repeat mutations affected the ability of E1E2 to mediate viral entry into Huh7 cells. Fig. 3, A and B, indicates that Pro and Ala substitution of all but one heptad repeat residue (Ser678) abolished viral entry competence, irrespective of an effect on E1E2 heterodimerization. In the case of Ser678, the Pro mutation blocked both E1E2 heterodimerization and viral entry competence, whereas S678A was associated with the incorporation of wild type levels of E1E2 heterodimer into entry-competent virus.

Mutations in the Heptad Repeat Region of E2 Do Not Affect CD81 Binding—The CD81 tetraspanin is a critical component of the cellular receptor complex utilized by E1E2 for viral entry (16, 17). We therefore determined whether mutated HCV glycoproteins derived from HIV-1 pseudotypes retained CD81 binding ability. Viral lysates were titrated against a recombinant form of the CD81 large extracellular loop, MBP-LEL113–201, in an enzyme-linked immunosorbent assay (4).
Fig. 4. Binding of E1E2-HIV-1-pseudotyped particle lysates containing proline (A) or alanine (B) mutations to recombinant LEL of CD81 (MBP-LEL113–201). Leu675 (∆), Ser678 (×), Leu682(○), Leu685(●), Leu689(□), Ile692(△), Val699 (○), WT (●), and particles lacking E1E2 (■). Bound HCV glycoproteins were detected using mAb H53 and rabbit anti-mouse horseradish peroxidase in an enzyme immunoassay.

Fig. 5. A, heterodimerization of E1E2 proline mutations in cell lysates. Metabolically labeled lysates of 293T cells transfected with wild type (WT), mutated pE1E2H77c, or empty pcDNA4 vector (no E1E2) were immunoprecipitated with the conformation-dependent anti-E2 mAb H53. The immunoprecipitants were examined by nonreducing SDS-PAGE on 10–15% polyacrylamide gradient gels. E1 and E2 were visualized by phosphorimage analysis. B, E1 expression in WT and mutated pE1E2H77c-transfected cells. Cell lysates were subjected to SDS-PAGE in 12% polyacrylamide gels prior to Western blotting with the E1-specific mAb A4. C, heterodimerization of E1E2 proline mutants in the context of HIV-1 pseudotypes. Metabolically labeled virus particles were pelleted from the tissue culture fluid prior to lysis in RIP buffer. E1E2 heterodimers were immunoprecipitated with mAb H53 prior to SDS-PAGE on 10–15% gradient gels and phosphorimage analysis. D, incorporation of HIV-1 structural proteins into E1E2-pseudotyped particles. Metabolically labeled virus particles were pelleted from the tissue culture fluid prior to lysis in RIP buffer. HIV-1 proteins were immunoprecipitated with IgG from an HIV-1-infected individual prior to SDS-PAGE on 7.5–15% gradient gels and phosphorimage analysis. E, entry of wild type (WT) and mutated E1E2-HIV-1 pseudotypes into Huh7 cells. The data represent the percentage of entry of mutant versus wild type (means ± S.E.; five independent experiments performed in quadruplicate). F, binding of E1E2-pseudotyped particle lysates to recombinant LEL of CD81 (MBP-LEL113–201). Bound HCV glycoproteins were detected using mAb H53 and rabbit anti-mouse horseradish peroxidase-conjugated antibody in an enzyme immunoassay. P676A (∗), P683A (■), WT (●), and particles lacking E1E2 (■).
Bound HCV glycoproteins were detected with the conformation-dependent anti-E2 mAb, H53. Lysates of E1E2-pseudotyped particles with mutations in the E2 heptad repeat retained comparable CD81 binding ability, although slight reductions in binding were observed for the L675P and S678P mutants (Figs. 4, A and B), which are incorporated at lower levels into particles (Fig. 2B). These results indicate that the heptad repeat mutations did not significantly alter the CD81 binding site of E2. The inability of E1E2-HIV-1 pseudotypes to enter Huh7 cells is therefore most likely related to defective heterodimerization for the L675P/A, S678P, L689P/A, and L692P/A mutants or a defect at a post-CD81 binding stage of entry, such as membrane fusion, for the remaining entry-incompetent mutants.

Role of Conserved Proline Residues in the Heptad Repeat Region of HCV E2—Located in all strains and genotypes of HCV E2 are two proline residues, Pro 676 and Pro 683, that occupy consecutive b positions of the hydrophobic heptad repeat (Fig. 1.). Mutation of either proline to alanine did not affect E1E2 heterodimer formation (Fig. 5A), E1 biosynthesis (Fig. 5B), or incorporation of E1E2 heterodimers into pseudotyped HIV-1 particles (Fig. 5C). Similar amounts of the HIV-1 structural proteins were incorporated into pseudotyped particles in all cases (Fig. 5D). However, the P683A mutation abolished the ability of E1E2-pseudotyped HIV-1 particles to enter Huh7 cells, whereas P676A did not significantly affect entry competence (Fig. 5E). The binding of wild type and mutated E1E2 derived from pseudotyped HIV-1 particles to recombinant CD81 was not affected by the Pro to Ala substitutions (Fig. 5F). These data indicate Pro 683 is critical for the ability of E1E2 to mediate viral entry at a post-CD81 binding step.

Contribution of the E1 and E2 TMDs to Glycoprotein Heterodimerization—Previous studies have pointed to a role for the TMDs of E1 and E2 in their heterodimerization because this function is blocked following truncation of the E2 TMD from an E1E2 polyprotein (residues 170–715) or by mutations in the E1 and E2 TMDs (23–25). Furthermore, heterodimerization is not observed for a chimeric glycoprotein comprising the E1 and E2 ectodomains linked to the yellow fever virus prM and E TMDs, respectively, but lacking residues 700–715 of the putative E2 stem (26). We therefore asked whether the E2
heptad repeat sequence could function as a determinant of heterodimerization in the presence of foreign TMDs. A comparison of the hydropathy plots of yellow fever virus polypeptide prM with that of HCV E1E2 reveals a high degree of similarity in the TMDs (Fig 6A). Together with sequence comparisons and TMD predictions (Ref. 27; data not shown), we inferred that the most favorable linkages for an HCV-yellow fever virus chimera are E1L117–337-prM247–293-E2381–715, E706–728 (Fig 6, A and B). Fig 6C shows that chimeric E1M and E2E glycoproteins based on these linkages maintain the ability to heterodimerize. Pulse-chase biosynthetically labeled E2E was communoprecipitated with E1 by the E1-specific mAb A4, whereas E1A was communoprecipitated with E2 by the E2-specific mAb A11. In addition, immunoprecipitation with the conformation-dependent mAb H53 revealed that a significant proportion of E1G2E heterodimers are associated noncovalently (Fig 6D). However, noncovalent heterodimerization for E1M2E2E was less efficient than for wild type E1E2 (Fig 6D), and when pseudotyped into HIV-1 luciferase reporter particles, E1G2E2E lacked the ability to mediate entry into Huh7 cells (data not shown). These data indicate that the E2 stem and the TMDs of E1 and E2 contribute to glycoprotein heterodimerization and viral entry function.

**DISCUSSION**

We have shown that the membrane-proximal heptad repeat of E2 is an important determinant of E1E2 heterodimerization and viral entry function. This region of E2 is functionally homologous to a heptad repeat present at the C terminus of the flavivirus glycoprotein E stem region, which is also important for prM-E heterodimerization. Proline and alanine substitution of the α and δ positions of the E2 heptad repeat revealed that Leuα776, Serδ777, Leuδ778, and Leuα779 are key residues involved in heterodimerization with E1. Interestingly, the L675P substitution was tolerated in intracellular, predominantly immature, glycoprotein forms (1), whereas the mutation blocked E1E2 heterodimerization in a viral context. This result suggests that the intracellular forms of L675P-E1E2 may contain a nonconative configuration that blocks exit of the glycoprotein complex from the endoplasmic reticulum. Alternatively, the requirement of Leuδ776 for heterodimerization may differ in intracellular versus mature glycoprotein forms. The effects of L675A, S678A, L689A, and L692A substitutions on E1E2 heterodimerization in a viral context were less severe than the corresponding Pro substitutions. Substitution of these residues with the small helix-forming residue Ala may reduce specific E1 contacts without affecting the helical structure of this region, with the remaining heptad repeat residues maintaining weak heterodimerization. By contrast, introduction of the helix-breaking/kinking residue Pro at these positions is likely to result in the loss of helical structure and heterodimerization capacity. Interestingly, all HCV genotypes and strains therein contain Proδ776 and Proα779 in consecutive b positions, placing them on the opposite face of an α-helix relative to the α and δ residues. The presence of Pro within helices often causes the helical axis to bend by an average of 26° (28), suggesting that the N-terminal portion of the E2 heptad repeat lacks helicity. We found that the P683A mutation blocked viral entry competence, whereas entry was unaffected for the P676A mutant; neither mutation affected heterodimerization. These data indicate that Proδ776 plays a critical structural role in the heptad repeat region that is essential for E1E2 to function in viral entry.

Heterodimerization of E1 and E2 was maintained when their ectodomains were chimerized with the TMDs of prM and E, respectively. However, additional C-terminal determinants of heterodimerization and glycoprotein function appear to be required because E1M2E2E heterodimers formed less efficiently than did E1E2 and HIV-1 pseuodotyped with the E1M2E2E chimera was not competent to enter Huh7 cells. Previous studies using a similar chimera, but lacking E2-stem residues 700–715 adjacent to the TMD, did not result in E1M2E2E heterodimerization (26). Deletion of residues 700–715 alters the hydropathy plot of the E1α776E2E chimera significantly from that of wild type E1E2 (data not shown), which may result in an aberrantly folded heptad repeat region that is unable to mediate heterodimerization. Together these data suggest that the native TMDs of E1 and E2 and the juxtaplacent membrane region of E2 contain additional heterodimerization determinants or provide a structural context in which the E2 stem can fold and function correctly. These results are consistent with the observation that truncation at the C terminus of flaviviral glycoprotein E reduces the efficiency of prM-E heterodimerization, with removal of the E TMD having the greatest effect on prM-E association (14).

With the exception of Serδ776 and Proα779, all heptad repeat residues were critical for the ability of E1E2 to mediate entry of pseuodotyped HIV-1 particles into the hepatic cell line Huh7, irrespective of their role in heterodimerization. Because the mutations did not affect binding to the large extracellular loop of CD81, we expect that the heptad repeat functions at a postreceptor binding step of entry such as membrane fusion. An indication of how the heptad repeat may function in viral entry is provided by the crystal structures of flavivirus E glycoproteins at the pH of membrane fusion (13, 30). In these structures, the fusion loop at the tip of domain II anchors the E trimer to the target membrane. Low pH-induced interdomain rotations within E place the C terminus of the ectodomain close to the base of a channel that is formed by domain II intersubunit contacts, extending toward the fusion loops. Modis et al. (30) suggested that the stem region packs into this channel, drawing the adjacent TMD and viral membrane toward the cellular membrane for fusion. This mechanism is analogous to that utilized by class I fusion glycoproteins, such as influenza virus HA2, HIV-1 gp41, and simian virus 5 F protein, to effect membrane apposition. In these cases, a triple stranded coiled coil projects N-terminal fusion peptide toward the cellular membrane for insertion. A C-terminal segment, adjacent to the TMD, then packs into grooves formed on the surface of the coiled coiled immature conformation, thus drawing the TMDs and viral envelope toward the site of fusion peptide insertion (For review, see Refs. 11, 29). The heptad repeat region of E2 may play a similar role in membrane apposition by packing back against membrane-bound E2E611 to draw the TMD toward the membrane-inserted fusion loop. This is the first study examining the functional role of E1 and E2 in viral entry and indicates that HCV E2 has mechanistic features in common with class II fusion proteins.

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