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The Human T-Cell Leukemia/Lymphotropic Virus Type I p12^{I} Protein Cooperates with the E5 Oncoprotein of Bovine Papillomavirus in Cell Transformation and Binds the 16-Kilodalton Subunit of the Vacuolar H^+ ATPase

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The human T-cell leukemia/lymphotropic virus type I (HTLV-I) induces T-cell leukemia and transforms human T cells in vitro. A recently identified protein with a molecular weight of 12,000 (12K) (p12^{I}), encoded by single- and double-spliced mRNAs transcribed from the 3′ end of the HTLV-I genome, has been shown to localize in the perinuclear compartment and in the cellular endomembranes. The p12^{I} protein exhibits significant amino acid sequence similarity to the E5 oncoprotein of bovine papillomavirus type 1 (BPV-1). Both proteins are very hydrophobic, contain a glutamine residue in the middle of a potential transmembrane region(s), and are localized in similar cellular compartments. Because of these observations, we investigated whether the p12^{I} resemblance to E5 correlated with a similarity in their biological behavior. We expressed the p12^{I} protein to evaluate its ability to functionally cooperate with the BPV-1 E5 oncoprotein and to bind to a cellular target of the E5 protein, the 16K component of the vacuolar H^+ ATPase. Cotransfection of the mouse C127 cell line with the p12^{I} and E5 cDNAs showed that although p12^{I} alone could not induce focus formation, it strongly potentiated the transforming activity of E5. In addition, the p12^{I} protein bound to the 16K protein as efficiently as the E5 protein. These findings might provide new insight for potential mechanisms of HTLV-I transformation and suggest that p12^{I} and E5 represent an example of convergent evolution between RNA and DNA viruses.

Human T-cell leukemia/lymphotropic virus type I (HTLV-I) induces adult T-cell leukemia (6, 15, 26, 32) and tropical spastic paraparesis–HTLV-I-associated myelopathy, a progressive myelopathy (7, 29, 34). The mechanism of HTLV-I transformation of human T cells in vitro (24, 26, 33) and in vivo is not fully understood. The HTLV-I pX region encodes two transactivating proteins, p40^{tax} and p27^{rex}; one of unknown function, p21^{rex} (5, 14, 17, 18, 37, 38); and at least three other proteins—p12^{I}, p13^{II}, and p30^{II}—from open reading frames I and II, respectively (3, 19, 20). The p40^{tax} protein is thought to play an important role in T-cell proliferation by transactivating directly the interleukin 2 (IL-2) and the α chain of the IL-2 receptor (IL-2R) promoters (13). Furthermore, p40^{tax} has been shown to transform NIH 3T3 and rat fibroblasts in vitro (16) and to immortalize human T cells in vitro (12). The more recently identified p30^{II}, p13^{II}, and p12^{I} proteins have been shown to reside in the nucleolar, the nuclear, and the endomembrane compartments of transfected cells (19). The spliced mRNAs for these proteins have been demonstrated in several HTLV-I-infected cell lines (1, 3, 20), in fresh peripheral blood mononuclear cells from HTLV-I-infected individuals (1, 20), and in in vitro HTLV-I-infected human macrophages (21), although translation of these mRNAs in the infected cells has not yet been demonstrated.

Computer analysis of the HTLV-I p12^{I} protein indicated some genetic similarity with a viral transforming protein, the bovine papillomavirus type 1 (BPV-1) E5 oncoprotein (2, 36). To investigate whether this restricted genetic similarity could help explain the p12^{I} function, we studied the biological activity of the p12^{I} protein, either alone or in association with the E5 protein, in a transforming assay on the C127 mouse cell line. Furthermore, the interaction of the p12^{I} protein with a cellular target for E5, the 16-kDa subunit of the H^+ vacuolar ATPase (9, 11, 23, 28), was also tested.

Analysis of the putative amino acid sequence of the p12^{I} protein (99 amino acids) revealed the existence of two potential transmembrane domains, LALTALLLFLLPPSDVSGLLL and ILSGLLFLLFLPLFFSLPLLL, which contained an LFLL repeated amino acid motif (Fig. 1). A stretch of approximately 20 amino acids, encompassing the second potential transmembrane domain of the p12^{I} protein, displayed 50% amino acid identity with a portion of the BPV-1 transforming E5 protein (36). This region in E5 contains two LFLL repeated motifs (Fig. 1) and a hydrophilic glutamine residue which is essential for the transforming activity of this small oncogenic protein (10, 22). To determine the functional significance of the structural resemblance between these two viral proteins, the biological activity of the HTLV-I p12^{I} protein was tested in a focus-forming assay on the C127 mouse cell line either alone or in combination with the E5 protein. In this assay, both the natural and the tagged (AU1) p12^{I} cDNAs (19, 39) were used. The p12^{I} protein did not induce focus formation, whereas the E5 protein did (Fig. 2). However, the ability of the E5 protein to induce focus formation was dramatically increased in a dose-dependent manner (Fig. 2) when the cDNA for the E5

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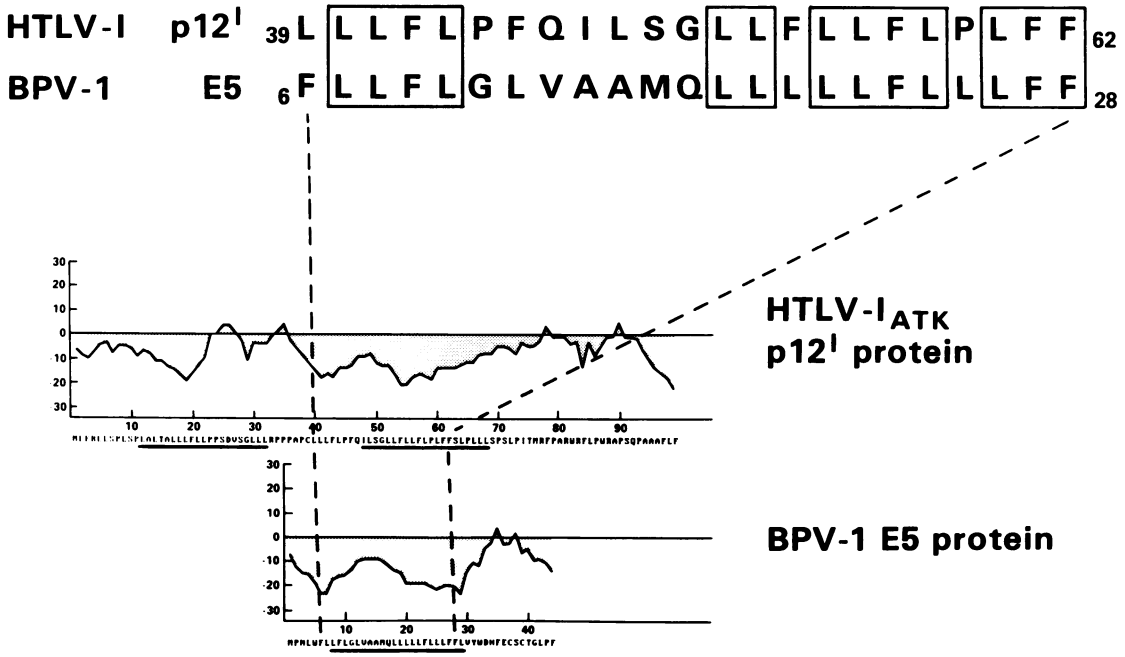

FIG. 1. Hydropathy profiles of the HTLV-I p12^{I} and BPV-1 E5 proteins and the amino acid alignment of a conserved region in the two proteins. The top panel shows a region of approximately 50% amino acid identity between p12^{I} and E5 (the single-letter amino acid code is used). The lower panel graphically represents the hydrophobic and hydrophilic portions of the proteins, with the putative transmembrane regions of both proteins underlined by a solid bar. The amino acid sequence of HTLV-I p12^{I} was derived from HTLV-I_{ATK}. This analysis was performed with the alignment program of Microgenie software.

and p12^{I} were cotransfected. A parallel experiment which used the plasmid HCMV-HSPA, containing a copy of the p12^{I} open reading frame which lacks the methionine initiating codon (p12$^{I}\Delta$ATG), failed to show this effect even when a high ratio (1:9) of E5 to p12$^{I}\Delta$ATG was used (Fig. 2).

To evaluate whether p12^{I} and E5 might interact with similar cellular proteins, we performed coprecipitation studies with epitope-tagged forms of p12^{I} and E5. The vector HCMV-HSPA was used to express epitope-p12^{I} fusion protein (p12$^{I}_{HA1}$), which could be detected with the monoclonal antibody (Ab) 12CA5 (19, 39). The pCMV-E5 constructs were used to express the HA1 epitope-E5 ($E5_{HA1}$) and the AU1 epitope-16K ($16K_{AU1}$) fusion proteins. The plasmid DNAs were transfected into HeLa-Tat cells, which were then metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine, and the proteins were extracted and immunoprecipitated with mouse monoclonal Ab against the HA1 epitope (12CA5) and the AU1 epitope (anti-AU1). As previously demonstrated (9), the E5 and 16K proteins were coimmunoprecipitated with monoclonal Ab directed against either the E5 tag (HA1) or the 16K tag (AU1) (Fig. 3A, lanes 7 and 8). The specificity of Ab recognition for the appropriate epitopes was demonstrated in Fig. 3A. The 12CA5 Ab, but not the AU1 Ab, specifically immunoprecipitated the E5-tagged protein (Fig. 3A, lanes 1 and 2). Similarly, the AU1 Ab specifically immunoprecipitated the 16K-tagged protein (Fig. 3A, lane 6). Neither Ab immunoprecipitated specific proteins in HeLa-Tat cells transfected with only the vector DNA (Fig. 3A, lanes 3 and 4).

As in the case of E5, when the p12$^{I}_{HA1}$ protein was coexpressed with the $p16K_{AU1}$ protein, coprecipitation of both proteins was observed with Ab against either of the respective tags (Fig. 3C, lanes 12 and 13). The p12^{I} interaction with the 16K component of the H^{+} vacuolar ATPase does not appear

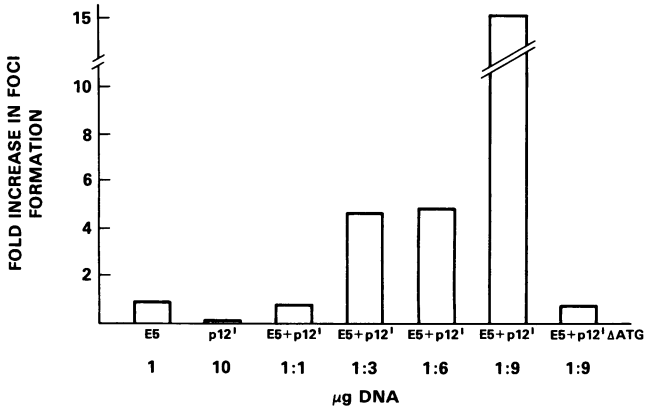

FIG. 2. The HTLV-I p12^{I} gene potentiates cellular transformation by E5 in C127 cells. The fold increase in focus numbers is represented on the *y* axis, and the ratio of E5 and p12^{I} DNA (in micrograms of DNA) is indicated under each histogram. A total of 5×10^{5} C127 cells were plated in 60-mm-diameter dishes. The next day cells were transfected by calcium phosphate precipitation overnight, glycerol shocked for 1 min, and seeded in a 100-mm-diameter dish 2 days later. The medium (Dulbecco modified Eagle medium plus fetal calf serum) was changed every 4 days, and after 1 month the dishes were stained with 1% methylene blue in ethanol for 30 min. Control HCMV-HSPA vector was used as a carrier DNA to normalize the amount of transfected DNA in each transfection. The two left histograms represent E5 (1 μg of the pJS21 plasmid) and $p12_{AU1}$ (10 μg) transfectants. The data presented in the figure were derived from experiments performed in duplicate. The average enhancement obtained in five different experiments with a ratio of E5 to p12^{I} of 1:9 μg was 8- to 10-fold.

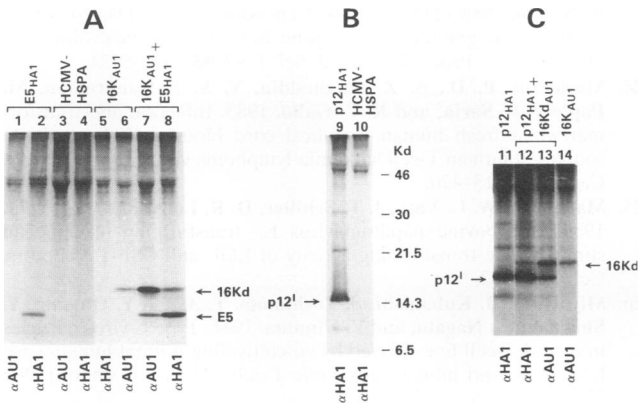

FIG. 3. Coprecipitation of the E5-16K and p12^{I}-16K proteins co-expressed in HeLa-Tat cells. Cells were transfected and immunoprecipitated as previously described (19). The 12CA5 Ab recognizes the HA1 epitope, and the AU1 Ab recognizes the homonymous epitope. The immunoprecipitates were loaded on either sodium dodecyl sulfate-polyacrylamide (15 or 18% acrylamide) denaturing gels. The proteins expressed by the cDNA in each transfection are indicated above each panel. At the bottom, the monoclonal Ab used in each lane is indicated.

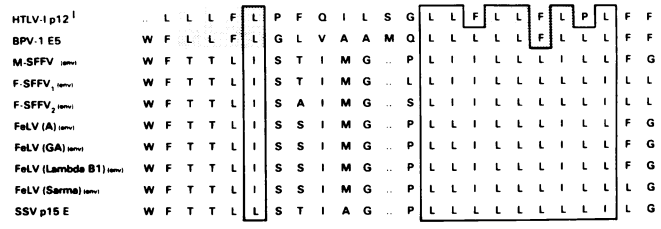

FIG. 4. Amino acid alignment of HTLV-I$_{ATK}$ p12^{I}, BPV-1 E5, and the carboxyl-terminal portion of the envelope glycoproteins of animal retroviruses. The amino acids are designated according to the single letter amino acid code. Boxes show regions of high-level amino acid conservation; shading indicates repeated motifs present only in p12^{I} and E5. M-SFFV, mink SFFV; F-SFFV, Friend SFFV; FeLV, feline leukemia virus; SSV, simian sarcoma virus.

to be due to simple hydrophobic interactions, since the p12^{I} protein failed to bind other proteins containing hydrophobic transmembrane domains, including the α chain of the IL-2R, the platelet-derived growth factor receptor (PDGFR), and the epidermal growth factor receptor (data not shown). Since 16K is almost entirely hydrophobic, it is more likely that p12^{I}-16K binding reflects specific interactions between protein domains. However, the definition of the interacting domains will require mutagenic analysis of both proteins. In addition to interacting with 16K, the p12^{I} protein was also observed to coprecipitate several additional cellular proteins (Fig. 3B and C, lanes 9 and 11 to 13) whose identities await further characterization.

Transformation of C127 cells by E5 appears to involve the activation of the PDGFR, and previous studies have suggested that this activation is the consequence of E5 binding to the PDGFR as a ligand (30, 31). The mechanism of cooperation between p12^{I} and E5 in the induction of C127 transformation is unclear at the present time. However, preliminary data suggest that these two viral proteins form heterodimers. Recent studies have also shown that E5 binds to the PDGFR and 16K proteins via hydrophobic, transmembrane sequences and that E5, 16K, and PDGFR exist as a tricomponent complex (8).

The E5 protein has also been shown to cooperate with other receptors, such as the epidermal growth factor and colony stimulating factor 1 (25). Preliminary data demonstrate that while the p12^{I} protein does not bind to PDGF, epidermal growth factor, the α chain of the IL-2R, or the erythropoietin receptor, it does bind to the β chain of the IL-2R (27). The functional and biological significance of this protein-protein interaction is not yet clear, but the binding of a retroviral protein to a cell surface receptor has been shown to be biologically relevant in the mouse erythroleukemia model. In this system, induced by the Friend spleen focus-forming virus (SFFV), the binding of the carboxyl-terminal 37 amino acids of the mutated Friend SFFV gp55 envelope glycoprotein to the erythropoietin receptor is a crucial event for in vitro and in vivo transformation (for reviews, see references 4 and 35). An alignment of the amino acid sequences conserved between the E5 protein, the p12^{I} protein, and the carboxyl-terminal portion of the mink SFFV and Friend SFFV envelope glycoproteins identifies a region which is redundant in leucine and/or isoleucine (Fig. 4). Furthermore, other retroviruses, such as the feline leukemia virus and the simian sarcoma virus, which cause leukemia in cats and sarcoma/leukemia in monkeys, respectively, share the same conserved region at the carboxyl-terminal region of the envelope glycoproteins. This signature sequence may define a particular class of viruses which use binding to a relevant cellular receptor as a step in the mechanism of neoplastic transformation. The possible role of the 16K H^{+} ATPase subunit in the HTLV-I and the BPV-1 transformation pathways remains to be clarified.

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