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Human Immunodeficiency Virus *vpr* Product Is a Virion-Associated Regulatory Protein

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The *vpr* product of the human immunodeficiency virus type 1 (HIV-1) acts in *trans* to accelerate virus replication and cytopathic effect in T cells. Here it is shown that the HIV-1 viral particle contains multiple copies of the *vpr* protein. The *vpr* product is the first regulatory protein of HIV-1 to be found in the virus particle. This observation raises the possibility that *vpr* acts to facilitate the early steps of infection before de novo viral protein synthesis occurs.

The *gag*, *pol*, and *env* genes of human immunodeficiency virus type 1 (HIV-1) encode the structural and replicative proteins that are incorporated into the virus particle. HIV-1 specifies at least six additional proteins which regulate viral replication (Fig. 1). Two of these genes, *tat* and *rev*, are essential for virus replication. The remaining genes, *nef*, *vif*, *vpu*, and *vpr*, are not required for virus replication, although mutations in these genes alter the replication properties of the virus (3). *vpr* was recently demonstrated to accelerate the replication and the cytopathic effect of HIV-1 in $CD4^+$ T cells (2, 5). *vpr* was also shown to specify a 15-kilodalton (kDa) protein that acts in *trans* to increase expression of viral proteins. *vpr* also stimulates expression of heterologous genes driven by the HIV-1 long terminal repeat (LTR) as well as other promoters (2).

To determine whether the *vpr* protein is incorporated into the virus particle, $CD4^+$ T cells were transfected with infectious proviruses isogenic except for the expression of *vpr*. Cells were transfected by the DEAE-dextran technique (8). The provirus that expresses the full-length *vpr* protein of 96 amino acids is designated $HXBRU^+$. A provirus isogenic except for a frameshift mutation in *vpr* that is predicted to terminate the product at amino acid 40 was used as a control ($HXBRU^-$) (2).

Eight days posttransfection, both cultures were metabolically labeled with 100 μCi of [^{35}S]cysteine per ml for 8 h as described elsewhere (9). Cells were collected by centrifugation, washed once with phosphate-buffered saline, and lysed with 750 μl of a buffer which contained 0.05 M Tris hydrochloride (pH 7.0), 0.15 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% sodium dodecyl sulfate. The medium containing the virus particle was centrifuged at 800 × *g* and passed through a 0.22-μm-pore-size filter to prevent cell contamination of the supernatant. Cell-free supernatants were then centrifuged for 1 h at 16,000 rpm in an Eppendorf microcentrifuge.

Lysates were prepared from both the labeled transfected cells and from the virus pellets. The cell and viral lysates were immunoprecipitated as described previously (9) with antiserum from a patient with acquired immune deficiency syndrome (AIDS) which recognized a broad spectrum of viral structural proteins. The lysates were also precipitated with a monospecific anti-*vpr* serum. The rabbit *vpr* antiserum used was raised against a synthetic peptide corresponding to the amino-terminal 19 amino acids of the *vpr* protein. As described previously (2), this antiserum recognizes a 15-kDa protein in cells producing the *vpr* protein (Fig. 2A, lane 1). The peptide used to raise the antiserum competed for recognition of the 15-kDa protein (lane 2). The *vpr* product was not detected in cells producing the $HXBRU^-$ virus (lane 4).

The *vpr* peptide antiserum also recognized the 15-kDa protein in the lysates of pelleted virus prepared from cells producing the $HXBRU^+$ virus (Fig. 2B, lane 1). No *vpr* product was detected in viruses pelleted from cultures producing the $HXBRU^-$ virus (Fig. 2B, lane 3). In addition, a protein of approximately 9 kDa of molecular mass was detected only in viruses pelleted from cultures producing the $HXBRU^+$ and $HXBRU^-$ viruses with both the *vpr* peptide antiserum and the AIDS patient serum (Fig. 2B, lanes 1 to 4). The recognition of this 9-kDa protein was not competed for by the *vpr* peptide, suggesting a nonspecific reaction (data not shown). These results indicate that the *vpr* protein is associated with the virus particle. The association appears to

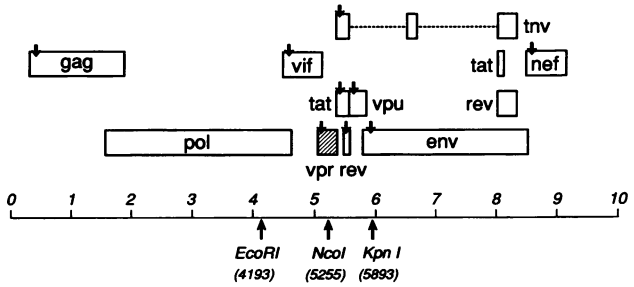

FIG. 1. Genetic organization of HIV-1. Arrows indicate the initiator AUG codons in viral genes. To generate a vpr^+ provirus, a segment of the HXBc2 provirus (6) located between the C terminus of *pol* (*Eco*RI at nucleotide position 4193; +1 is the site of initiation of transcription) and the N terminus of *env* (*Kpn*I site at nucleotide 5893), a region that contains the entire *vpr* coding sequence (▨), was replaced with the corresponding segment derived from the closely related BRU provirus (10). The genotype of the resultant provirus ($HXBRU^+$) is 5′ LTR_{HXBc2} gag^+_{HXBc2} pol^+_{HXBc2} vif^+_{BRU} vpr^+_{BRU} tat^+_{BRU} rev^+_{BRU} vpu^+_{BRU} env^+_{HXBc2} nef_{HXBc2} 3′ LTR_{HXBc2}. Introduction of a frameshift at an *Nco*I site (nucleotide position 5255) in $HXBRU^+$ generated a provirus expressing a truncated *vpr* product ($HXBRU^-$) (2). The location of the recently identified coding sequences of the *tat-env-rev* (*tnv*) fusion protein is shown (7a).

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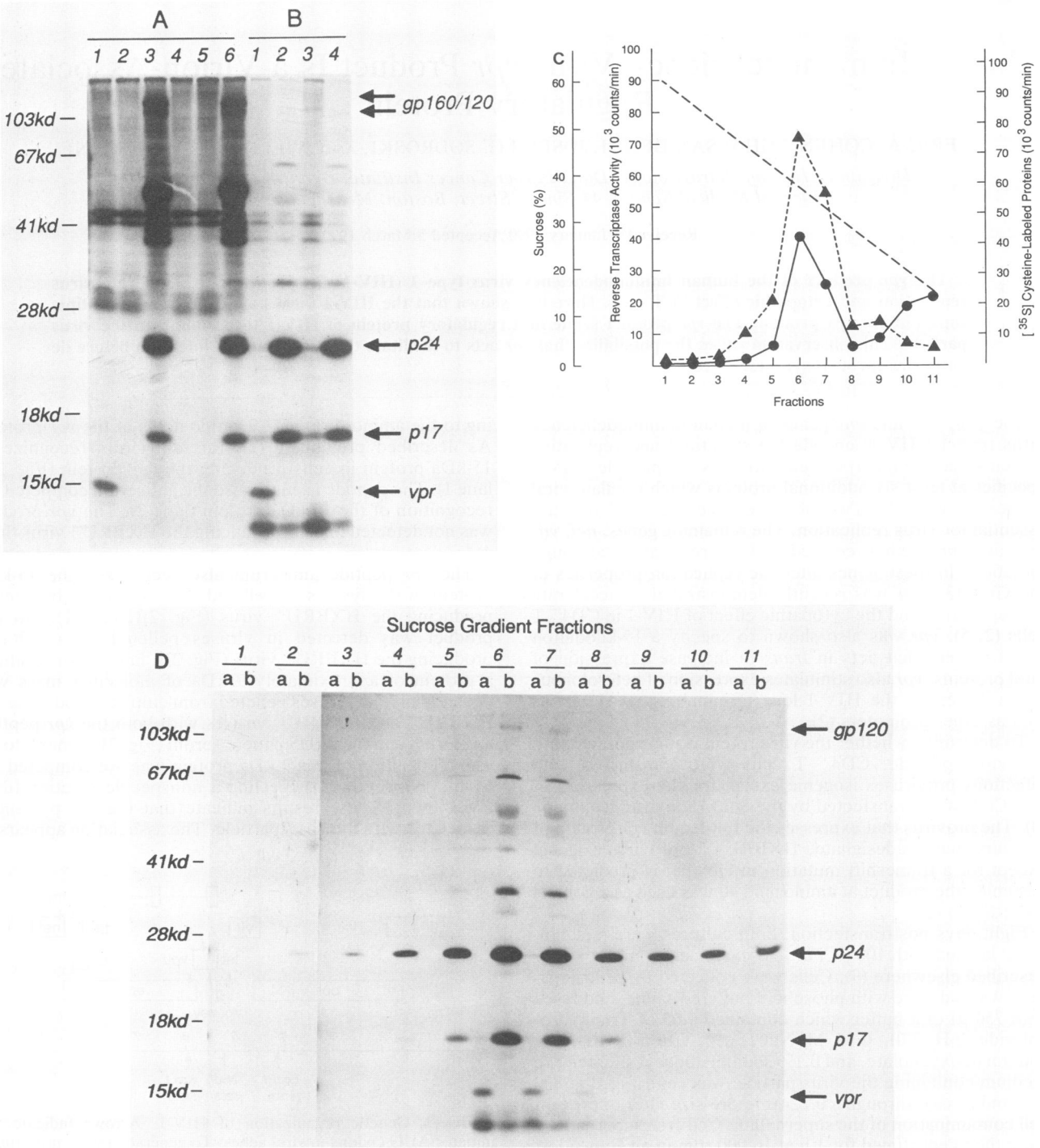

FIG. 2. Detection of the *vpr* product in the extracellular virions. (A) Lysates prepared from [^{35}S]cysteine-labeled Jurkat cells transfected with either HXBRU$^+$ (lanes 1 to 3) or HXBRU$^-$ (lanes 4 to 6) provirus were immunoprecipitated with *vpr* peptide antiserum (lanes 1 and 4), *vpr* peptide antiserum preincubated with 500 μmol of peptide (lanes 2 and 5), or AIDS patient serum (lanes 3 and 6). (B) Lysates of pelleted virus prepared from Jurkat cells transfected with either HXBRU$^+$ (lanes 1 and 2) or HXBRU$^-$ (lanes 3 and 4) provirus were immunoprecipitated with *vpr* peptide antiserum (lanes 1 and 3) or AIDS patient serum (lanes 2 and 4). (C) Pelleted virions prepared from [^{35}S] cysteine-labeled HXBRU$^+$-transfected Jurkat cells were suspended in 400 μl of TNE, layered on a 15 to 60% (wt/vol) sucrose gradient, and centrifuged at 45,000 rpm for 3 h as described previously (1). Eleven fractions (400 μl each) were collected. Reverse transcriptase assays (7) were performed on 50-μl portions of each fraction (▲). Ten microliters of each fraction was counted in a liquid scintillation counter to determine the amount of [^{35}S]cysteine-labeled proteins (●). (D) Immunoprecipitates of either 240 or 100 μl of each sucrose gradient fraction with *vpr* peptide antiserum (a) or AIDS patient antiserum (b), respectively. kd, Kilodaltons.

be specific, since in similar experiments using this and other proviruses, monospecific antiserum to the *tat*, *rev*, *vpu*, and *nef* proteins did not detect these proteins in the pelleted fraction of the viruses (data not shown).

To rule out cell contamination in the supernatant, the suspended HXBRU$^+$ virus pellet was layered onto a sucrose gradient as described previously (1). The virus was suspended in 400 μl of a buffer (TNE) which contained Tris (10 mM; pH 7.4), 100 mM NaCl, and 1 mM EDTA. A 4-ml linear sucrose gradient (15 to 60%, wt/vol) spun at 45,000 rpm for 3 h was used. Eleven fractions of 400 μl each were collected. The amount of [^{35}S]cysteine-labeled protein in each fraction and the reverse transcriptase activity were determined (Fig. 2C) (7). Additionally, each fraction was precipitated with either an AIDS patient antiserum or the monospecific *vpr* antiserum (Fig. 2D). The *vpr* protein cosedimented with virus particles by all measures used (Fig. 2C and D). The peak of *vpr* protein corresponds to the peak of reverse transcriptase activity, the peak of [^{35}S]cysteine-labeled proteins in the gradient, and the peak of virion proteins as detected by AIDS patient antiserum.

The amount of radioactivity in the bands corresponding to p24, p17, and *vpr* p15 protein in the immunoprecipitates of cell lysates and virus particles was determined. The results showed that the ratios of the proteins associated with the viral particles and the cells were roughly equivalent, between 5:1 and 10:1 (data not shown). Although it is difficult to calculate stoichiometry from such measurements because the efficiency of immunoprecipitation may vary for each protein, the results indicate that multiple *vpr* proteins were incorporated into each virion.

The results of these experiments demonstrate that the *vpr* protein is incorporated into cell-free virus particles. The *vpr* protein is the first regulatory product of HIV-1 to be found associated with viral particles. Experiments similar to these failed to detect the *tat*, *rev*, *vpu*, or *nef* protein associated with virus particles (E. A. Cohen, J. G. Sodroski, and W. A. Haseltine, unpublished observations). The *vpx* protein of HIV type 2 and of the simian immunodeficiency virus was also recently reported to be associated with the virus particles (12). There is no similarity in amino acid sequence between the *vpr* and *vpx* proteins.

The mechanism by which *vpr* becomes associated with the virus particle is unknown. Other capsid proteins are made as *gag* or *gag-pol* precursors and assembled as units into the budding particle (4, 11). The *vpr* protein is not known to be part of such a larger precursor and must have an independent means of association with the nascent particle.

The presence of a transactivating protein in the mature virus particles suggests that this protein plays a role in early steps of virus infection. Possible roles for such a protein include facilitation of the reverse transcription reaction, stabilization of RNA, stabilization of RNA-DNA or DNA-DNA structures, facilitated migration of the proviral DNA to the nucleus, and facilitated integration. The ability of the *vpr* protein to increase the rate of expression of the HIV-1 and other promoters raises the possibility that the virion-associated *vpr* may activate early transcription of the HIV-1 provirus. The *vpr* product may also alter cellular expression before viral RNA transcription begins. Alteration of the cell environment by *vpr* carried into the cell by the infecting virus may alter the initial rate of HIV-1 RNA accumulation before viral proteins are made de novo.

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