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Integrase of Human Endogenous Retrovirus K-10 Supports the Replication of Replication-Incompetent *Int*⁻ Human Immunodeficiency Virus Type 1 Mutant

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The integration of proviral DNA is an essential step for retroviral replication and is mediated by a viral integrase (IN). In general, IN utilizes, as a donor substrate, long terminal repeats (LTR) of homologous origin but not of heterologous origin (1). However, in vitro studies suggested that human endogenous retrovirus (HERV) K-10 IN could utilize not only the homologous LTR but also human immunodeficiency virus type 1 (HIV-1) LTR as a substrate (4). This report demonstrates that *Int*-negative HIV-1 mutant replicated in cells simultaneously expressing HERV IN. This suggests that the HERV IN could utilize LTR of HIV-1, which is only distantly related to HERV (2).

Vpr-PC (protease cleavage sequence)-IN fusion protein has been shown to be incorporated into virions and cleaved into VprPC and IN during virion maturation (5,9). Therefore, we constructed a vector which expressed VprPC fused to IN of HERV (pC-VprPC/HERVIN) (Fig. 1). To determine whether HERV IN protein could be incorporated into HIV-1 virions, we transfected 293T cells (3) with pC-VprPC/IN together with pHQHY, an HIV-1 clone encoding non-functional mutated IN, which had base substitutions in the 12th and 16th histidine residues (6). Immunoblot analysis (Fig. 2) revealed the presence of HERV IN in HIV_{HQHY} virions released from the transfected 293T cells, suggesting that HERV IN was actually incorporated into HIV-1 virions. Similarly, we prepared

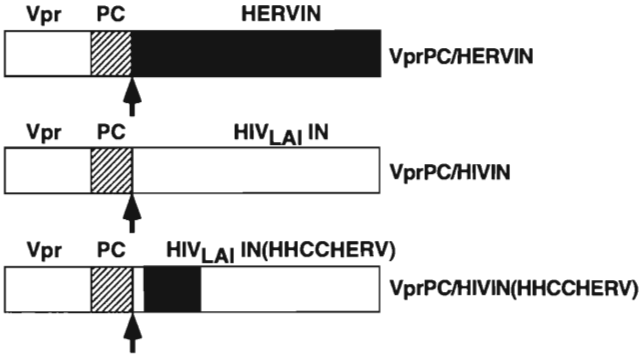

Fig. 1. Schematic representation of VprPC fusion proteins.
The left open boxes, Vpr of HIV_{LAI}; dashed boxes, a linker peptide containing the influenza virus hemagglutinin (HA) and a junctional peptide of RT and IN (from refs. 5, 9); closed boxes, integrase of HERV K-10; open boxes, integrase of HIV_{LAI}.

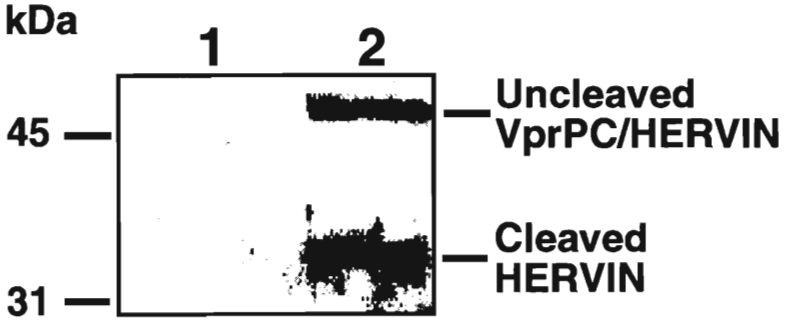

Fig. 2. Immunoblot analysis of virion proteins.
Virions were collected by centrifugation, and lysed in RIPA buffer. Virion proteins were separated in a 15% polyacrylamide gel and blotted onto a nitrocellulose membrane. The bound proteins were probed with anti-HERVIN rabbit serum which was not cross-reactive to HIV-1 IN and then with anti-rabbit HRP-conjugated goat serum, and visualized using an ECL plus kit (Amersham Pharmacia Biotech, AB., Uppsala, Sweden). Analyzed are $NL4\text{-}3_{HQHY}$ virions released from 293T cells in the presence of $VprPC/HIV_{LAI}IN$ (lane 1) or VprPC/HERVIN (lane 2). The size markers (31 and 45 kDa) are positioned alongside the left of the gel. HIV protease partially cleaved the incorporated VprPC/HERVIN (50 kDa) to generate VprPC (18 kDa) and HERV IN (32 kDa) (Fig. 1).

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Table. Infectivities of the virions incorporating various INs

	Control[a]	HIV_{LAI} IN[b]	HERV IN[b]	HHCCHERV[b]
Nunber of blue cells[c]	302±2.94	60±1.63	2.2±0.81	0±0.00

[a] Wild-type HIV-1 (strain LAI) released from the 293T cells transfected with the infectious clone DNA (pLAI).

[b] Virions released from the 293T cells transfected with DNA of an integration-defective HIV-1 mutant (HQHY) along with pC-VprPC/HIVIN, pC-VprPC/HERVIN, or pC-VprPC/HHCCHERVIN.

[c] MAGIC cells (4×10^4 cells/well in a 24-well plate) were infected with each sample (equivalent to 1 ng of p24). The cells were stained with X-gal for cellular β-galactosidase expression two days post-infection. The blue-stained cells were counted. Results obtained from five independent experiments were presented as 'average ± standard deviation.'

HIV_{HQHY} virions that incorporated the intact IN of $HIV\text{-}1_{LAI}$ as a positive control and those which incorporated non-functional HERV IN, HIVIN(HHCCHERV) (amino acid residues 17-39 of HIV IN were replaced with 23 amino acid residues, VNAAGLKNKFDVTWKQAKDIVQH) as a negative control.

We compared infectivities of the virions incorporating various INs in MAGIC cells (7,8). The inocula contained the same amount of p24. The virions that incorporated the intact HERV IN actually produced blue colonies, and their estimated infectivity was about 3.7% of the infectivity of the virions incorporating the intact HIV-1 IN (Table). As the in vitro integration activity of HERV IN was about 10-30% of HIV-1 IN when HIV-1 LTR was used as a donor substrate (4), the value of 3.7% may be reasonable. Virions incorporating $HIVIN_{HHCCHERV}$ showed no detectable infectivity. These experiments suggest that HERV IN mediated the integration reaction of HIV-1 LTR in vivo.

The above experiments may raise the possibility of an interaction between human endogenous retrovirus and HIV-1 during the replication process. In light of this, the development of integrase negative HIV mutant should be performed with caution.

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