SUPPLEMENTARY MATERIALS AND METHODS LEBLANC et al

Plasmids

The human PrP-GPI+ and PrP-ΔGPI (hereafter named HumPrP-GPI+ and HumPrP-ΔGPI) encoding full length and ΔGPI PrP proteins are expressed under a CMV promoter from a pCRUni vector (Invitrogen). The murine PrP-GPI+ and PrP-ΔGPI (hereafter named MurPrP-GPI+ and MurPrP-ΔGPI) constructs are provided by Sylvain Lehmann and are expressed under a CMV promoter from a pcDNA3 vector (Invitrogen).

Western blotting

Cell lysis, protein extraction and Western blotting procedures have been previously described (Leblanc et al., 2004). Signals were quantified by scanning the autoradiographs using the personal densitometer SI (Molecular dynamics).

Virus production by DNA transfection.

NIH3T3-22L cells were plated at 1.2x10^6 cells in 100 mm plates one day before transfection. Cells were transfected with the lipofectamine Plus™ Reagent kit (Invitrogen) according to the manufacturer instructions. The amounts of transfected plasmids used in the different experiments were: MoMuLV-GagPol (pGPP), 8µg; MoMuLV-Env (FBMoSALF), 8µg and MoMuLVGagPol+Env (4 µg pGPP and 4 µg FBMoSALF).

MoMuLV-WT proviral DNA (pNCS), 8 µg, MoMuLV-Δp12 (pNCS-Δp12): 8 µg and MoMuLV-ΔDPPPY (pNCS-ΔDPPPY): 8 µg.

MoMuLV-WT proviral DNA (pRR88): 8 µg and MoMuLV-ΔNC(16-23) (pRR88-ΔNC(16-23)): 8 µg.

293T cells were plated at 3x10^6 cells in 100 mm plates one day before transfection. Cells were cotransfected by calcium phosphate method with the HIV-1 pNL4-3 (10 µg) proviral genome and
HumPrP-GPI+ or HumPrP-ΔGPI expressing constructs (10 µg). Similar experiments were realized with the pNCS MoMuLV proviral genome and the murine PrP expressing constructs MurPrP-GPI+ and MurPrP-ΔGPI. Virions were recovered 48 h after transfection.

**Sucrose density gradient centrifugation**

The 100,000g pellet obtained after the differential centrifugation steps was resuspended in PBS and layered onto a 10-60% linear sucrose gradient. Centrifugation was for 16 h at 100,000g at 4°C in a SW41 rotor. Fractions (x18) were recovered from the top and RT activity and densities were determined. Each fraction was analyzed by Western blotting using anti-Env gp70, anti-CAp30, anti-PrP and anti-EF1α antibodies.

**Detergent Resistant Microdomains (DRMs) isolation**

DRMs from MoMuLV-infected NIH3T3 cells were isolated as previously described (Leblanc et al., 2004). Cells were washed and scraped with ice-cold PBS. Pelleted cells were lysed on ice for 20 min in TNE (25 mM Tris HCl pH7.5; 150 mM NaCl; 5 mM EDTA) containing 1% Triton X-100 and protease inhibitor cocktail. The cell lysate was diluted in 60% sucrose (wt/wt) to obtain a 45% sucrose concentration in 3 ml. The lysate was overlaid with 6 ml of 30% TNE sucrose and 3 ml of 5% TNE sucrose in an SW41 Ti ultracentrifuge tube. The step gradient was centrifuged for 20 h at 200,000g at 4°C. Eleven 1 ml fractions (excluding the pellet) were collected from the top of the gradient. 30 µl of each fraction was analyzed by dot immunoassay using anti-PrP, anti-CAp30, anti-gp70 antibodies or the biotinylated cholera toxin for the GM1 raft marker. PrPSc detection in DRMs was carried out as previously described (Vey et al., 1996).

**Virus capture**

Recruitment of the murine PrPc into MoMuLV virions was examined according to a published protocol (Bounou et al., 2001; Bounou et al., 2002). The anti-mouse magnetic beads (Dynabeads
Pan Mouse IgG from Dynal) were coated with the anti-PrP SAF-32 antibodies or the anti-CAp24 directed against the capsid of HIV-1 as a negative control. Coated beads were incubated with clarified and filtered viral supernatant from NIH3T3i cells in binding buffer (1X PBS, 0.1% BSA) for 1 h at 4°C. Beads were washed three times with binding buffer and resuspended in binding buffer containing 1.5% of Triton X-100 for viral particles lysis. The amount of immunocaptured MoMuLV virions was assessed by RT detection.

**Electron microscopy**

Immunogold labeling on ultrathin cryosections: cells were fixed with a mixture of 2% paraformaldehyde (PFA) and 0.125% glutaraldehyde in 0.2 M phosphate buffer pH 7.4 for 2 h at room temperature. Cells were processed for ultrathin cryosectionning, immunogold-labeled and contrasted as described in Février et al. PrP was detected with a mixture of PrP antibodies (SAF32 and SAF83), MoMuLV virions with CAp30 antibodies and HIV-1 virions with CAp24 or Envgp120 antibodies. Anti-PrP and -CAp30, –EF1α or -CAp24 antibodies were recognized with PAG coupled to 15 nm (PAG15) and 10 nm (PAG10) gold particles, respectively.

Immunogold labeling of viral particles and exosomes: MoMuLV virions and exosomes were recovered from the NIH3T3-22L-MoMuLV supernatant after concentration through a 20% sucrose cushion at 100,000g in a SW28 rotor. The pellet was gently resuspended and fixed with a mixture of 2% PFA, 0.065% glutaraldehyde in 0.2M phosphate buffer at 4°C. 10 µl suspension was loaded on formvar-carbon-coated electronic microscopy grids and fixed a second time. Some grids were permeabilised with 0.1% saponin during 15 min for the detection of CAp30/Gag and EF1a localized inside the viral particles or the exosomes, respectively. To detect PrPSc, the fixed samples were treated with or without denaturing buffer (3M guanidine isothiocyanate, 50mM Tris HCl pH7.5) during 5 min. After extensive washing, single and double immunoGold labeling
were realized using the anti-PrP (SAF83), -CAp30 or -EF1α antibodies followed by addition of PAG 15 and PAG10 for colabeling.

Similar conditions were used for isolated HIV-1 viral particles. Virions were recovered from the 293T-PrP-HIV-1 cell supernatant 48 h after transfection, concentrated through a 20% sucrose cushion and resuspended in phosphate buffer. Once fixed and loaded onto grids, double immunogold labeling was realized using anti-PrP (SAF37) and anti-Envgp120 antibodies followed by PAG 15 and 10, respectively.

Samples were contrasted and embedded in a mixture of uranyl acetate and methylcellulose and viewed under a CM120 electron microscope (Philips, Eindhoven, The Netherlands).