Supplementary methods data

Cells

HEK293 cells in which the expression of Agno was inducible by Dox (293AG cells) were established with the T-REx system (Invitrogen). To establish 293AG cells stably expressing LBR-EGFP, we transfected 293AG cells with pLBR-EGFP; G418-resistant colonies were isolated and the expression of LBR-EGFP was confirmed by immunoblot analysis.

Antibodies

A mouse monoclonal antibody to HP1α was from Chemicon. Rabbit polyclonal antibodies to JCV Agno and VP1 were generated as described (Okada *et al.*, 2002) and those to EGFP were kindly provided by N. Mochizuki. A horseradish peroxidase–conjugated mouse monoclonal antibody to Myc was from Invitrogen. Mouse monoclonal anti-NPC antibody was purchased from Covance Research Products. Anti-Lamin a/c antibody was from Santa Cruz Biotechnology.

Transfection, immunoblot analysis, and immunoprecipitation

Transfection was performed with the use of Lipofectamine 2000 (Invitrogen). For immunoblot analysis, cells were lysed in RIPA buffer and immune complexes were detected with horseradish peroxidase–conjugated secondary antibodies (with the exception of anti-Myc) and ECL reagents (Amersham Pharmacia).

Immunoprecipitaiton was performed using fractionated cell lysates. Cell fractionation was carried out by the procedure exploiting density properties for separation of nucleus and ER from other membrane fractions as previously described (Michael E. Greenberg, 1997) with slight modifications. In brief, 2 - 10 x 10^6 cells were resuspended in ice-cold sucrose buffer I (0.32 M sucrose, 3 mM calcium chloride, 2 mM magnesium acetate, 0.1 mM EDTA, 10mM Tris-HCl, pH 8.0, 1mM DTT, and 0.5% Nonidet P-40), and then added sucrose buffer II (1.8 M sucrose, 5 mM magnesium acetate, 0.1 mM EDTA, 10 mM Tris-HCl, pH 8.0, 1mM DTT). The lysates were gently overlaid onto sucrose buffer II in open-top centrifuge tubes to make discontinuous sucrose gradients. The gradients were centrifuged at 30,000 g for 45 min at 4°C. After centrifugation, supernatants were removed and the pellets were washed with PBS containing 0.5% Triton X-100. The samples were lysed in nuclear lysis buffer (25 mM Tris-HCl, pH 7.4, 300 mM of sodium chloride, 0.5% Nonidet P-40, and 0.5% sodium deoxycholate), and then rotated for 1 h at 4°C for complete extraction. After rotation, the lysates were centrifuged at 20,000 g for 30 min at 4°C, and the supernatant were applied for immunoblotting and immnoprecipitation. For immunoprecipitation, the lysates were precleaned by incubation with protein A-Sepharose FF beads (Amersham Pharmacia) for 1 h, and incubated with antibody-coupled protein A beads for 4 h at 4°C. Following six cycles of washing with RIPA buffer or wash buffer (50 mM Tris-HCl, pH 7.5, 100 mM sodium chloride, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.02% BSA, and 0.5 mM

phenylmethylsulfonyl fluoride), the precipitates were subjected to SDS-PAGE.

Ref: Michael E. Greenberg, T.P.B. (1997) Identification of Newly Transcribed RNA. *Current Protocols in Molecular Biology,* **Unit 4.10**.

Immunofluorescence analysis

Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS), permeabilized with 0.1% Triton X-100 in PBS, and incubated with 5% dried skim milk in PBS at room temperature. They were then incubated with primary antibodies, after which immune complexes were detected with Alexa 488– or Alexa 594–labeled goat antibodies to rabbit immunoglobulin G or with Alexa 594–labeled goat antibodies to mouse immunoglobulin G (Molecular Probes). Nuclei were stained with propidium iodide. Cells were observed with a confocal laser-scanning microscope (Olympus).

Yeast two-hybrid assay

The Matchmaker System 3 and HEK293 cell cDNA library were obtained from Clontech. A cDNA encoding the NH_2-terminal 24 amino acids of JCV Agno (N24) was subcloned into the pGBKT7 yeast shuttle vector (Clontech). Yeast AH109 cells were cotransformed with pGBKT7-agnoN24 and cDNAs from the HEK293 cell library. Plasmids isolated from positive colonies were introduced into *Escherichia coli* DH5α and sequenced; the DNA sequence data were compared with sequences in the NCBI database with the BLAST program.

Microinjection of VLPs

JC VLPs composed of VP1 were purified as described (Komagome et al., 2002; Qu et al., 2004; Suzuki et al., 2001). 293AG cells were incubated with or without Dox for 24 h before microinjection of nuclei with a mixture of unlabeled VLPs (1.8 mg/ml) and Cy3 (1 mg/ml) with the use of a glass capillary. The cells were then incubated for 60 min at 37°C, washed with PBS, and fixed with 3.7% formaldehyde. VLPs were detected by immunostaining with anti-VP1 and fluorescein isothiocyanate–conjugated secondary antibodies, and fluorescence signals were photographed with a cooled charge-coupled device camera system (Olympus).