JOURNAL OF VIROLOGY, Apr. 1996, p. 2562–2568 Vol. 70, No. 4
0022-538X/96/$04.00+0

NOTES

The Epstein-Barr Virus-Encoded Nuclear Antigen EBNA-5 Accumulates in PML-Containing Bodies

LASZLO SZEKELY,[1]* KATJA POKROVSKAJA,[1] WEI-QIN JIANG,[2] HUGUES DE THE,[3,4] NILS RINGERTZ,[2] AND GEORGE KLEIN[1]

Microbiology and Tumor Biology Center[1] and Department of Cell and Molecular Biology,[2] Karolinska Institute, S-171 77 Stockholm, Sweden, and Centre National de la Recherche Scientifique UPR 43, Hôpital St. Louis 1,[3] and Service de Biochimie,[4] 75475 Paris Cedex 10, France

Received 14 November 1995/Accepted 11 January 1996

EBNA-5 is one of the Epstein-Barr virus (EBV)-encoded nuclear proteins required for immortalization of human B lymphocytes. In the nuclei of EBV-transformed lymphoblastoid cell lines EBNA-5 is preferentially targetted to distinct nuclear foci. Previously we have shown (W. Q. Jiang, L. Szekely, V. Wendel-Hansen, N. Ringertz, G. Klein, and A. Rosén, Exp. Cell Res. 197:314–318, 1991) that the same foci also contained the retinoblastoma (Rb) protein. Using a similar double immunofluorescence technique, we now show that these foci colocalize with nuclear bodies positive for PML, the promyelocytic leukemia-associated protein. Artificial spreading of the chromatin by exposure to the forces of fluid surface tension disrupts this colocalization gradually, suggesting that the bodies consist of at least two subcomponents. Heat shock or metabolic stress induced by high cell density leads to the release of EBNA-5 from the PML-positive nuclear bodies and induces it to translocate to the nucleoli. In addition to their presence in nuclear bodies, both proteins are occasionally present in nuclear aggregates and doughnut-like structures in which PML is concentrated in an outer shell. Nuclear bodies with prominent PML staining are seen in resting B lymphocytes. This staining pattern does not change upon EBV infection. In freshly infected cells EBNA-5 antigens are first distributed throughout the nucleoplasm. After a few days intensely staining foci develop. These foci coincide with PML-positive nuclear bodies. At a later stage and in established lymphoblastoid cell lines EBNA-5 is almost exclusively present in the PML-positive nuclear foci. The colocalization is restricted to EBV-infected human lymphoblasts. The data presented indicate that the distinct EBNA-5 foci are not newly formed structures but the result of translocation of the viral protein to a specialized domain present already in the nuclei of uninfected cells.

Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) express six nuclear proteins (EBNA-1 to -6) and three membrane proteins (LMP1, 2A, and 2B). Six of the nine proteins, including the protein examined in the present study, EBNA-5, are required for efficient immortalization (5, 20, 32). Together with EBNA-2, EBNA-5 is one of the earliest nuclear proteins expressed in EBV-infected B cells (1, 2). These two EBNAs can drive the resting B lymphocyte, the prime target of EBV infection, into the G_1 phase of the cell cycle (26).

Levels of EBNA-5 expression are highest during the first days of EBV infection but decline later (11). In freshly infected cells, EBNA-5 is homogeneously distributed throughout the nucleoplasm. After several days of infection EBNA-5 appears in distinct nuclear foci (29). In established LCLs the focal pattern dominates. The homogeneous nucleoplasmic staining decreases or disappears completely (15, 24).

Previously we have found that the nuclear foci that contain EBNA-5 in LCLs also contain retinoblastoma (Rb) protein (14). We also demonstrated that EBNA-5 is able to bind both Rb and p53 proteins in vitro (30). High-level expression of EBNA-5 in freshly infected B cells coincides with the increased transient expression of p53 and augmented expression of Rb in the same cells (29). The nuclear distribution of EBNA-5 prompted us to compare its immunofluorescence staining pattern with those of other nuclear proteins showing a focal expression pattern. The literature contains many reports of such patterns and of nuclear bodies (for reviews, see references 3 and 31) of unknown function. The present study was carried out with the aim of examining the distribution of EBNA-5 in relation to bodies containing the PML (promyelocytic leukemia-associated) protein.

The gene encoding the PML protein was identified as the fusion partner of retinoic acid receptor α in the t(15:17) translocations of acute promyelocytic leukemias (7, 16). The PML protein preferentially localized to distinct nuclear bodies or PODS, whereas the leukemia-associated fusion protein is distributed in a micropunctated fashion (9). Treatment of acute promyelocytic leukemias with retinoic acids restores the punctated staining pattern (6, 17, 33).

Nuclear bodies, corresponding to the normal PML staining pattern, were already identified decades ago, first by electron microscopy (8) and later by immunostaining with sera from patients with autoimmune conditions like primary biliary cirrhosis (18). Many of these sera react with PML itself.

Proteins encoded by DNA viruses have previously been found to accumulate in PML bodies. The ICP0 protein of herpes simplex virus, which contributes to the initiation of the immediate-early viral protein cascade (21, 23), is targeted to the PML- and Sp100-containing nuclear foci. Cotransfection of ICP0 with ICP4, the major immediate-early transcriptional

* Corresponding author. Phone: 46 8 728 67 60. Fax: 46 8 32 78 75. Electronic mail address: lassze@ki.se.

FIG. 1. Colocalization between EBNA-5 (green) and PML (red) in IB4 nuclei. The calculated overlap is labelled white. Bar, 5 μm.

regulator of herpes simplex virus type 1, has led to the entrapment of ICP4 in the ICP0-containing bodies (23). Infection with adenoviruses induces an extensive redistribution of the PML from the spherical nuclear bodies to filamentous structures. The protein encoded by *E4-ORF3* is responsible for this reorganization. Furthermore, it was demonstrated that the E1A oncoprotein of adenovirus concentrates in the PML-positive nuclear bodies (4).

In the present study, we show that EBNA-5 is accumulated in the PML-containing bodies both in freshly infected B cells and in established LCLs but disappears from these structures upon heat shock or metabolic stress or during chromatin spreading by surface tension.

EBNA-5 and PML in LCLs. In order to establish whether the EBNA-5 (14)-and PML-containing nuclear bodies (8, 9) were identical, lymphoblastoid cells were stained with anti-EBNA-5 mouse monoclonal and anti-PML rabbit polyclonal antibodies. Immunofluorescent staining was carried out in the following way. Cells were centrifuged on glass slides in a Cytospin centrifuge at 1,000 rpm for 5 min and fixed with methanol-acetone (1:1). The slides were rehydrated in phosphate-buffered saline for 30 min, washed in balanced salt solution, and incubated with the antibodies diluted in a blocking buffer (2% bovine serum albumin plus 0.02% NaN_3 in balanced salt solution). The slides were washed five times in balanced salt solution after the individual incubation steps (regularly 30 to 60 min at room temperature).

The following antibodies were used: JF186, anti-EBNA-5 mouse monoclonal antibody (11), anti-PML rabbit polyclonal serum (6), fluorescein isothiocyanate-conjugated Fab2 fragment of goat anti-mouse immunoglobulin (Ig) (Pierce), and Texas red-conjugated goat anti-rabbit Ig (Vector). The secondary conjugates were further absorbed onto acetone-fixed IB4 cells (13). Double fluorescence stainings of EBNA-4 and PML were carried out in the following order: anti-EBNA-5 monoclonal antibody (1:40), anti-PML (1:500), fluorescein isothiocyanate-conjugated anti-mouse Ig (1:20), normal mouse serum (1:50), and Texas red-conjugated anti-rabbit Ig (1:30). The cross-reactions were excluded by replacing the primary antibodies with isotype-identical anti-simian virus 40 heat-labile enterotoxin monoclonal antibody and normal rabbit serum, respectively, in parallel reactions. The images were recorded on a DAS microscope (Leitz DM RB) with a Hamamatsu dual-mode cooled charge-coupled device camera (C4880) and analyzed on a Pentium personal computer using Image Pro-Plus (Media Cybernetics) and Adobe Photoshop programs.

Most of the studies were carried out on IB4 cells, an LCL that contains one integrated copy of the EBV genome and uses the *W* viral promoter to initiate the giant message from which all the EBNAs are spliced. IB4, unlike most other LCLs, expresses high levels of EBNA-5. In IB4 EBNA-5 is localized to brilliantly stained nuclear bodies as well as, to various extents, to homogeneously stained nucleoplasm. In most LCLs EBNA-5 is restricted to the weakly or moderately stained nuclear bodies. PML staining showed no difference between IB4 and other LCLs. It detected 2 to 20 nuclear bodies, whereas the nucleoplasm remained essentially negative.

Figure 1 shows the results of double immunofluorescence staining and digital overlap analysis. There was a high degree of colocalization between PML- and EBNA-5-positive nuclear bodies in interphase IB4. Ninety-six percent of the nuclear bodies were detectably positive for both proteins. A similar colocalization was found in two other LCLs. These cell lines were established 6 and 9 months earlier by infecting CD19-selected peripheral blood B cells, obtained from healthy donors, with B95-8 virus.

The two colocalized nuclear proteins, EBNA-5 and PML, were usually assembled in 2 to 20 nuclear dots of various sizes

FIG. 2. EBNA-5 (A and D; green), PML (B and E; red), and overlap (C and F; white) in nuclear aggregates (A to C) and doughnut-like structures (D to F) in IB4 cells. Bar, 5 μm.

and shapes. In most cases these structures were spherical, but occasionally they appeared as doughnut-like structures, aggregates of nuclear bodies, or thread-like structures. Although the locations of EBNA-5- and PML-positive bodies were identical, the relative staining intensities of EBNA-5 and PML in individual dots varied. High-resolution examination of the larger aggregates and the doughnut-like structures showed that the distribution of EBNA-5 within these bodies might be quite different from that of PML. PML appeared to form an outer shell, whereas EBNA-5 was located inside these structures (Fig. 2).

PML and EBNA-5 in spread chromatin. In order to study the relationship between the two colocalized proteins further, the chromatin was spread by the surface tension of a fluid drop with rapidly increasing surface area, according to the method of Schlammadinger (25) with the modification that prefixation was omitted and methanol-acetone (1:1) was used as a fixative. In this procedure the chromatin forms a series of scaffold structures from which chromatin loops radiate laterally. Double immunofluorescence staining showed that the PML-containing bodies did not change their size or shape upon chromatin spreading. They remained preferentially localized within the area of the nuclear scaffold. In contrast, the EBNA-5-containing dots became more numerous and tended to be associated with more-peripheral chromatin loops. As a result, the extent of colocalization between EBNA-5 and PML decreased with increasing degrees of chromatin spreading (Fig. 3).

Heat shock disrupts the colocalization between EBNA-5 and PML. We have recently shown that heat shock or metabolic stress induced by high cell density causes the reversible translocation of EBNA-5 to the nucleolus in IB4 and other cells (28). Now we demonstrate that a brief heat shock (30 min at 45°C) leads to the complete dissociation of EBNA-5 from the PML bodies. The PML bodies remain in the nucleoplasm, and at the same time there is an almost complete relocation of EBNA-5 into the nucleoli (Fig. 4).

EBNA-5 and PML dissociate during mitosis. The number and the staining intensity of PML-positive nuclear bodies vary with the cell cycle (18). We found that all interphase nuclei showed colocalization between EBNA-5 and PML; consequently, the interphase position does not affect the colocalization. In contrast, the proteins dissociated during mitosis. PML preferentially accumulated in two to three intensely stained conglomerates within the area of the mitotic spindle, close to the chromosomes. EBNA-5 was distributed diffusely over the whole cytoplasm (Fig. 5).

EBNA-5 and PML in freshly infected B cells. In freshly infected B lymphocytes EBNA-5 appears as early as 12 h postinfection. It is diffusely distributed in the whole nucleoplasm during the early hours but starts appearing in nuclear bodies as well by the end of the first day (29). Uninfected B cells contained intensely stained PML-positive nuclear bodies. That distribution remained unchanged after EBV infection. The nascent EBNA-5 bodies colocalized with the PML-positive structures from the earliest time at which they became visible. Several days after infection, the EBNA-5 staining became completely restricted to distinct nuclear dots, showing perfect overlap with the PML-positive bodies (Fig. 6).

Colocalization between EBNA-5 and PML is restricted to EBV-infected human B lymphoblasts. Expression of EBNA-5 from a retrovirus-based vector in CV1 monkey kidney epithelial cells, SW480 human colon carcinoma cells, and Saos-2 human osteosarcoma cells leads to the accumulation of EBNA-5 in the nucleoplasm in an almost homogeneous fashion. In addition, a fraction of the cells (around 20%) also

FIG. 3. Disruption of colocalization by spreading of the chromatin. The extent of colocalization decreased in parallel with the increase in the extent of spreading. The extent of spreading was calculated by dividing the area of the measured object by the area of an average unspread nucleus. Moderate spreading (4.8-fold; A to C) only slightly affected the colocalization. Further spreading (5.2-fold; D to F) showed clear differences in the staining pattern, whereas extensive spreading (19-fold; G to I) led to the dissociation of EBNA-5- and PML-positive structures. EBNA-5 (A, D, and G; green), PML (B, E, and H; red), overlap (C, F, and I; white), and DNA (blue) are shown. Bar, 5 μm.

FIG. 4. Disruption of colocalization by 30-min heat shock. PML staining (A), a phase-contrast image (B), EBNA-5 staining (C), and a composite image of EBNA-5 (green) and PML (red) in IB4 nuclei (blue) (D) are shown. A three-color overlap image of PML, EBNA-5, and DNA of a single nucleus (E) shows that upon heat shock EBNA-5 is dissociated from the PML bodies and accumulates in the nucleolus. The bar in panel C represents 20 μm (scale for panels A to D), and that in panel E represents 5 μm.

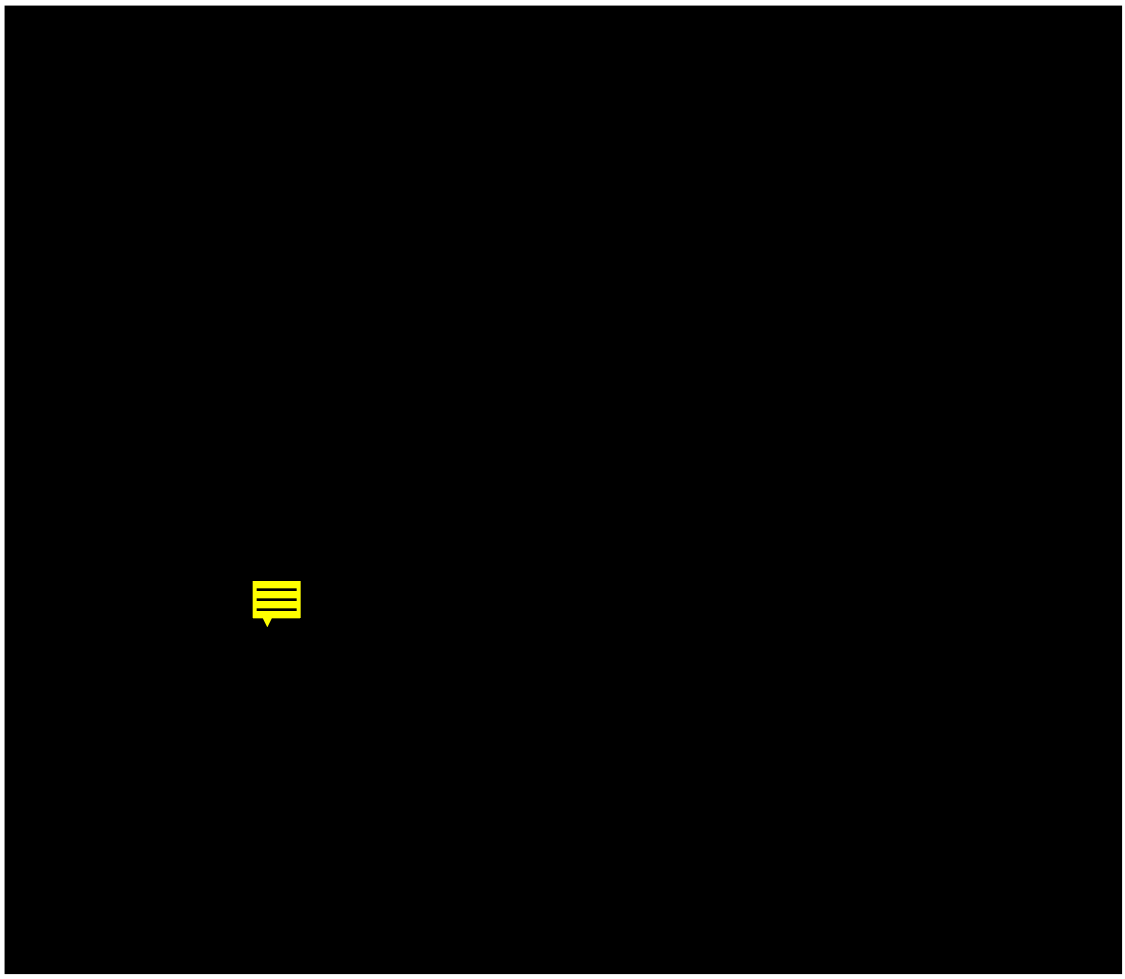


FIG. 5. Dissociation of EBNA-5 and PML during mitosis. The colocalization is still preserved in the prophase nucleus (A to C); however, a cell in metaphase (D to F; arrow in panels D and E) contains PML in large cytoplasmic bodies, whereas EBNA-5 is homogeneously distributed all over the cytoplasm. Neighboring interphase cells show perfect colocalization. EBNA-5 (A and D; green), PML (B and E; red), overlap (C and F; white), and DNA (blue) are shown. The bar in panel A represents 10 μm (scale for panels A to C), and that in panel D represents 5 μm (scale for panels D to F).

contain brilliantly stained nuclear bodies that contain Hsp70 heat shock protein as well (29). All of these cells contained PML bodies. Interestingly, the EBNA-5-positive foci showed no colocalization with the PML bodies in any of the transfectants. Moreover, EBNA-5-transfected EBV-negative DG75 Burkitt lymphoma cells, which express EBNA-5 antigens as fine speckled nuclear dots, failed to show colocalization with PML. We also tested the virus-producing cell line B95-8, a marmoset LCL. PML showed well-defined nuclear bodies in these cells, whereas EBNA-5 was present in numerous nuclear dots that failed to colocalize with PML.

The PML-positive nuclear bodies are novel organelles that contain at least four, but presumably many more, different proteins (9). The disruption of these structures in promyelocytic leukemias by the expression of the PML-retinoic acid receptor fusion protein appears to play a key role in cell transformation (17, 33). The function of PML and these nuclear bodies so far remains unknown. Previous reports have suggested that PML bodies play a role in host cell resistance to viral infection and/or in the regulation of gene expression (9). PML-containing nuclear bodies appear to be important targets of interferon response. Three proteins, i.e., PML, Sp100, and NDP52, are regulated by alfa and gamma interferon (12, 19, 27). The PML protein itself may act as a tumor suppressor gene product (22).

Lytic infection of cells by DNA viruses like herpes simplex virus, cytomegalovirus, and adenovirus leads to the accumulation of viral regulatory proteins in the PML-containing nuclear bodies and eventually induces redistribution of the PML protein (10). Now we report that a latency-associated nuclear protein of EBV, EBNA-5, also accumulates in the PML bodies at the early phase of the infection and persists in these structures in established LCLs. Unlike the lytic-phase-associated proteins of other viruses, however, EBNA-5 does not induce morphological changes in the distribution of PML. Our findings also suggest that the previously detected EBNA-5–Rb-positive blobs are not newly formed structures. Rather, EBNA-5 accumulates in already-existing nuclear bodies.

The fact that EBNA-5 positive nuclear bodies in different transfected cells do not colocalize with PML argues that other cellular and/or viral factors that are present only in EBV-infected B lymphoblasts are required for the accumulation of EBNA-5 in the PML bodies.

Both PML and EBNA-5 are nuclear-matrix-associated proteins. The chromatin spreading experiments suggest that the two proteins associate with different elements of the matrix.

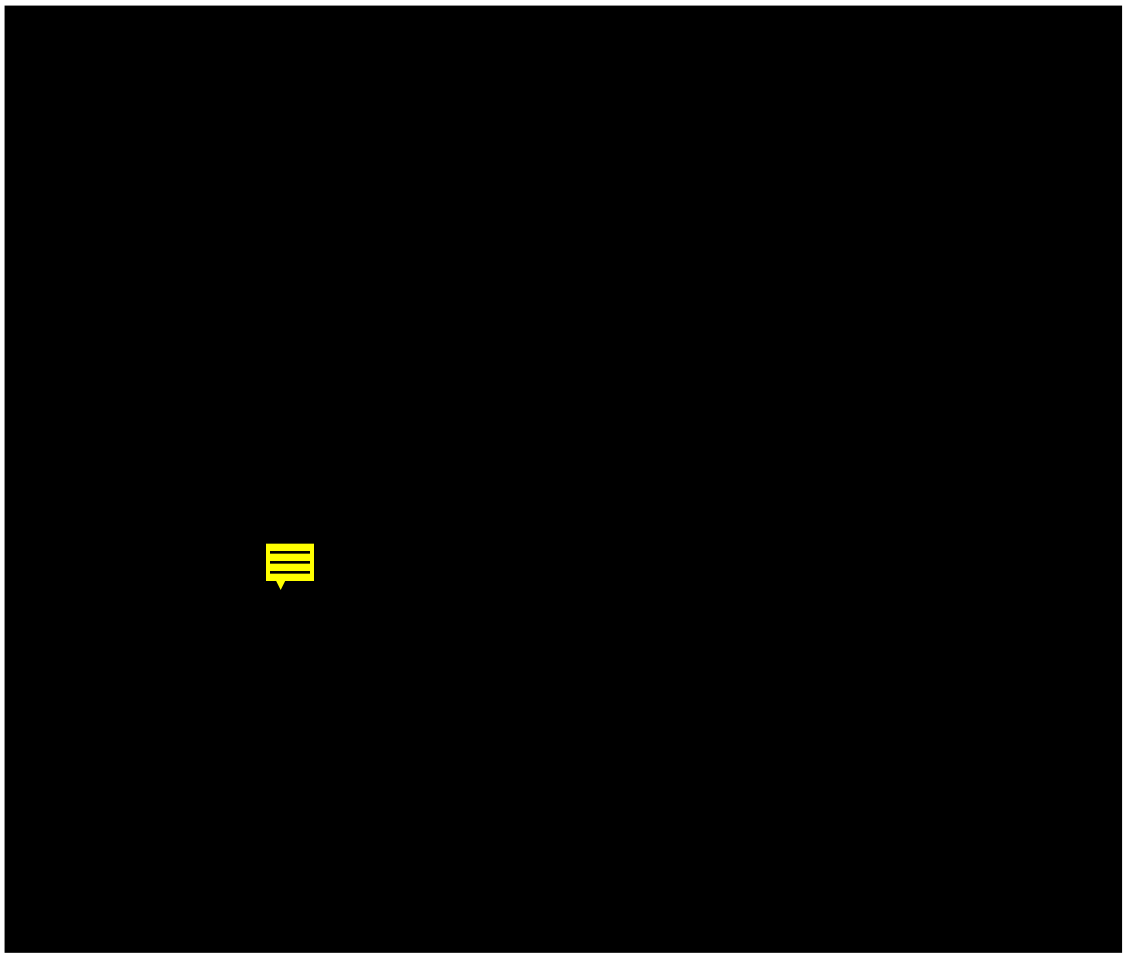


FIG. 6. EBNA-5 and PML in EBV-infected B cells after 72 h (A to C) and 8 days (D to F). EBNA-5 (green), PML (red), and overlap (white) are shown. Each bar represents 5 μm. That in panel A applies to panels A to C, and that in panel D applies to panels D to F.

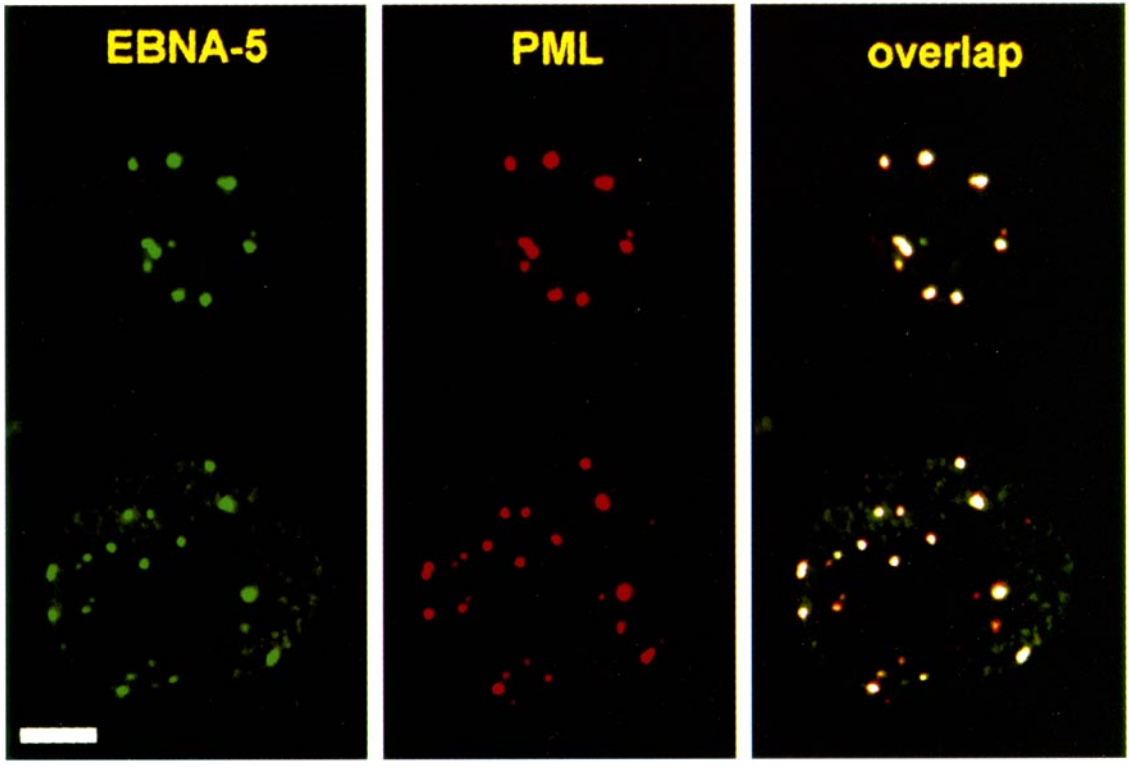
The PML antigen remains on the scaffold, whereas EBNA-5 is spread with the outpouring chromatin. On the basis of these findings, along with the differential localization patterns of the two proteins in the doughnut-like structures, we propose the following theory. PML is part of the outer shell of the nuclear body, firmly anchored to the nuclear scaffold. EBNA-5, on the other hand, accumulates inside the body but still preserves the connection to elements of the nuclear matrix that are linked more intimately to the chromatin than to the rigid scaffold.

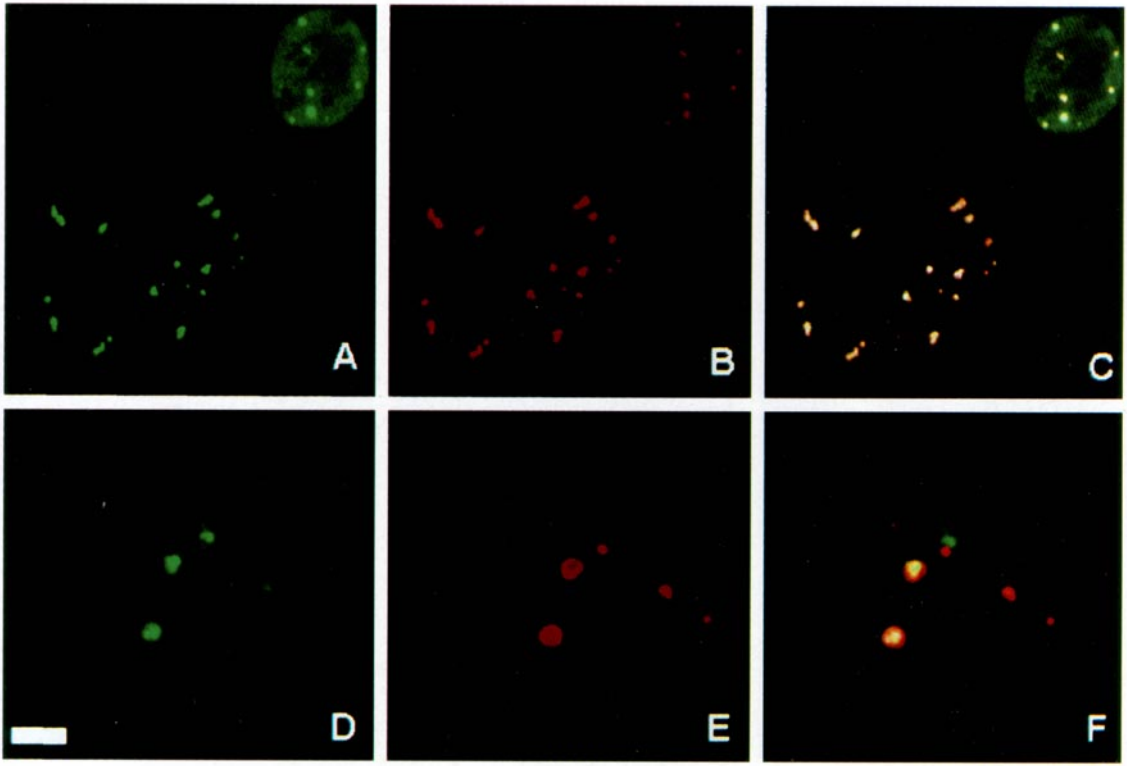
This work was supported by PHS grant 2 R01 CA14054-19 awarded by the National Cancer Institute, Department of Health and Human Services, and grants from the Swedish Cancer Society (Cancerfonden), the Swedish Medical Research Council, and the Concern Foundation/ Cancer Research Institute.

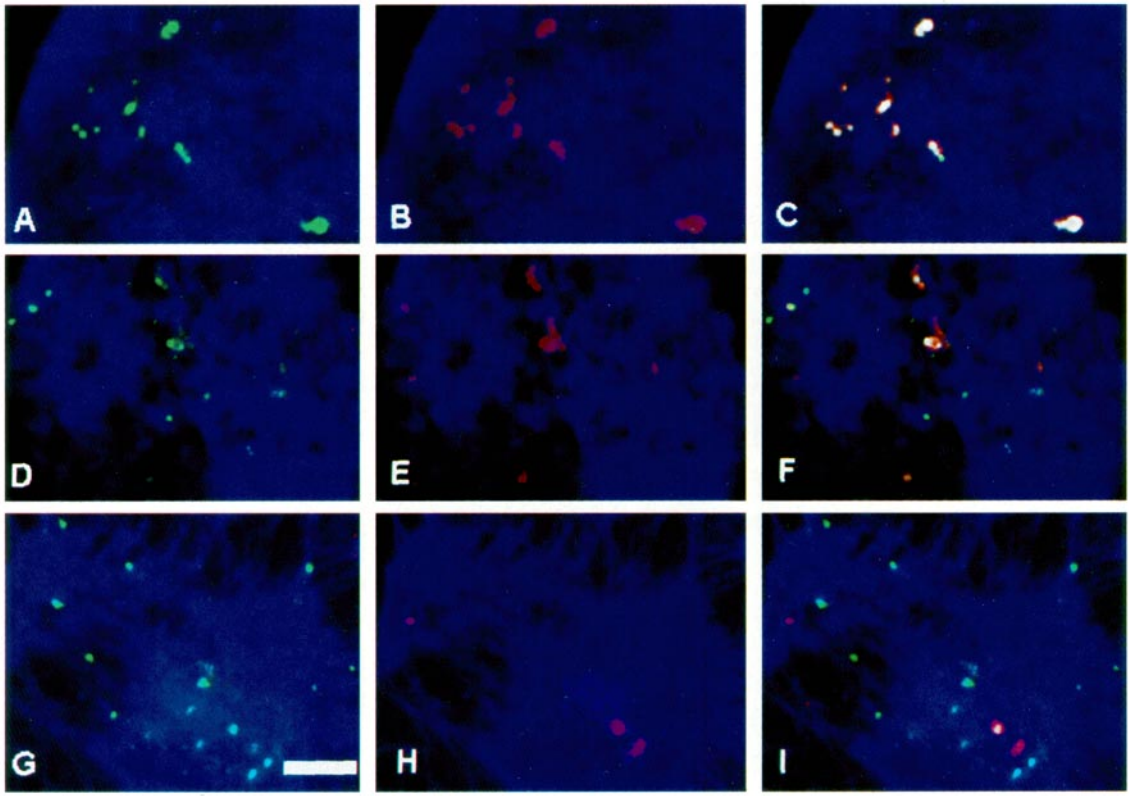
REFERENCES

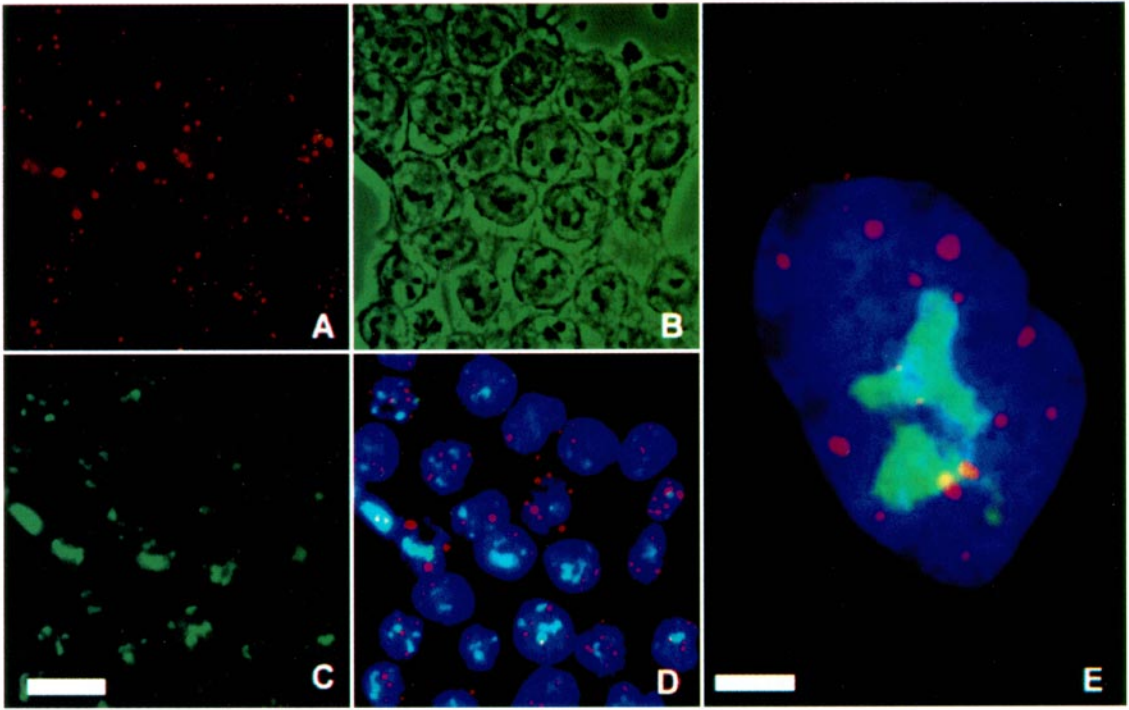
1. **Alfieri, C., M. Birkenbach, and E. Kieff.** 1991. Early events in Epstein-Barr virus infection of human B lymphocytes. Virology **181:**595–608. (Erratum, **185:**946.)
2. **Allday, M. J., D. H. Crawford, and B. E. Griffin.** 1989. Epstein-Barr virus latent gene expression during the initiation of B cell immortalization. J. Gen. Virol. **70:**1755–1764.
3. **Brasch, K., and R. L. Ochs.** 1992. Nuclear bodies (NBs): a newly "rediscovered" organelle. Exp. Cell Res. **202:**211–223.
4. **Carvalho, T., J. S. Seeler, K. Ohman, P. Jordan, U. Pettersson, G. Akusjarvi, M. Carmofonseca, and A. Dejean.** 1995. Targeting of adenovirus E1a and E4-ORF3 proteins to nuclear matrix-associated PML bodies. J. Cell Biol. **131:**45–56.
5. **Cohen, J. I., F. Wang, J. Mannick, and E. Kieff.** 1989. Epstein-Barr virus nuclear protein 2 is a key determinant of lymphocyte transformation. Proc. Natl. Acad. Sci. USA **86:**9558–9562.
6. **Daniel, M. T., M. Koken, O. Romagne, S. Barbey, A. Bazarbachi, M. Stadler, M. C. Guillemin, L. Degos, C. Chomienne, and H. de The.** 1993. PML protein expression in hematopoietic and acute promyelocytic leukemia cells. Blood **82:**1858–1867.
7. **de The, H., C. Lavau, A. Marchio, C. Chomienne, L. Degos, and A. Dejean.** 1991. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell **66:**675–684.
8. **de The, H., M. Riviere, and W. Bernhard.** 1960. Bull. Cancer **47:**570–584.
9. **Dyck, J. A., G. G. Maul, W. H. Miller, Jr., J. D. Chen, A. Kakizuka, and R. M. Evans.** 1994. A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell **76:**333–343.
10. **Everett, R. D., and G. G. Maul.** 1994. HSV-1 IE protein Vmw110 causes redistribution of PML. EMBO J. **13:**5062–5069.
11. **Finke, J., M. Rowe, B. Kallin, I. Ernberg, A. Rosen, J. Dillner, and G. Klein.** 1987. Monoclonal and polyclonal antibodies against Epstein-Barr virus nuclear antigen 5 (EBNA-5) detect multiple protein species in Burkitt's lymphoma and lymphoblastoid cell lines. J. Virol. **61:**3870–3878.
12. **Guldner, H., C. Szostecki, T. Grotzinger, and H. Will.** 1992. IFN enhances expression of Sp100, an autoantigen in primary biliary cirrhosis. J. Immunol. **149:**4067–4073.
13. **Harlow, E., and D. Lane. (ed.).** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Jiang, W. Q., L. Szekely, V. Wendel-Hansen, N. Ringertz, G. Klein, and A. Rosén.** 1991. Co-localization of the retinoblastoma protein and the Epstein-Barr virus-encoded nuclear antigen EBNA-5. Exp. Cell Res. **197:**314–318.
15. **Jiang, W. Q., V. Wendel-Hansen, A. Lundkvist, N. Ringertz, G. Klein, and A. Rosén.** 1991. Intranuclear distribution of Epstein-Barr virus-encoded nuclear antigens EBNA-1, -2, -3, and -5. J. Cell Sci. **99:**497–502.

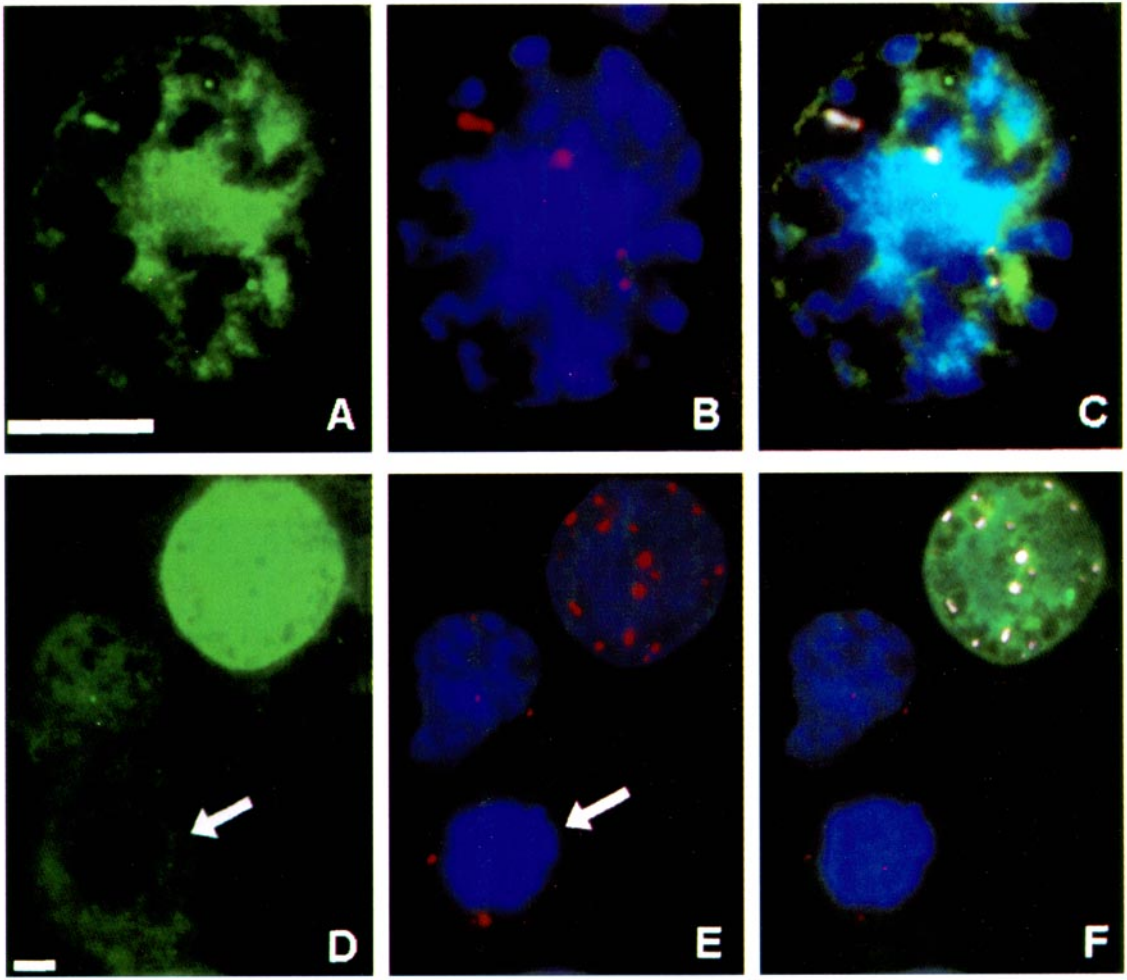
16. **Kakizuka, A., W. H. Miller, Jr., K. Umesono, R. P. Warrell, Jr., S. R. Frankel, V. V. Murty, E. Dmitrovsky, and R. M. Evans.** 1991. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. Cell **66:**663–674.
17. **Koken, M. H., F. Puvion-Dutilleul, M. C. Guillemin, A. Viron, G. Linares-Cruz, N. Stuurman, L. de Jong, C. Szostecki, F. Calvo, C. Chomienne, et al.** 1994. The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. EMBO J. **13:**1073–1083.
18. **Koken, M. H. M., G. Linares-Cruz, F. Quignon, A. Viron, M. K. Chelbi-Alix, J. Sobczak-Thepot, L. Juhlin, L. Degos, F. Calvo, and H. de The.** 1995. The PML growth-suppressor has altered expression in human oncogenesis. Oncogene **10:**1315–1324.
19. **Korioth, F., C. Gieffers, G. G. Maul, and J. Frev.** 1995. Molecular characterization of NDP52, a novel protein of the nuclear domain 10, which is redistributed upon virus infection and interferon treatment. J. Cell Biol. **130:**1–13.
20. **Mannick, J. B., J. I. Cohen, M. Birkenbach, A. Marchini, and E. Kieff.** 1991. The Epstein-Barr virus nuclear protein encoded by the leader of the EBNA RNAs is important in B-lymphocyte transformation. J. Virol. **65:**6826–6837.
21. **Maul, G. G., and R. D. Everett.** 1994. The nuclear location of PML, a cellular member of the C3HC4 zinc-binding domain protein family, is rearranged during herpes simplex virus infection by the C3HC4 viral protein ICP0. J. Gen. Virol. **75:**1223–1233.
22. **Mu, Z. M., K. V. Chin, J. H. Liu, G. Lozano, and K. S. Chang.** 1994. PML, a growth suppressor disrupted in acute promyelocytic leukemia. Mol. Cell. Biol. **14:**6858–6867.
23. **Mullen, M. A., S. Gerstberger, D. M. Ciufo, J. D. Mosca, and G. S. Hayward.** 1995. Evaluation of colocalization interactions between the IE110, IE175, and IE63 transactivator proteins of herpes simplex virus within subcellular punctate structures. J. Virol. **69:**476–491.
24. **Petti, L., C. Sample, and E. Kieff.** 1990. Subnuclear localization and phosphorylation of Epstein-Barr virus latent infection nuclear proteins. Virology **176:**563–574.
25. **Schlammadinger, J.** 1990. Immunofluorescence detection of nuclear antigens in supramolecular chromatin spreads. Methods Mol. Cell. Biol. **1:**235–241.
26. **Sinclair, A. J., I. Palmero, G. Peters, and P. J. Farrell.** 1994. EBNA-2 and EBNA-LP cooperate to cause G0 to G1 transition during immortalization of resting human B lymphocytes by Epstein-Barr virus. EMBO J. **13:**3321–3328.
27. **Stadler, M., M. K. Chelbi-Alix, M. H. M. Koken, L. Venturini, C. Lee, A. Saib, F. Quignon, L. Pelicano, M.-C. Guillemin, C. Schindler, and H. de The.** Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and GAS element. Oncogene, in press.
28. **Szekely, L., W.-Q. Jiang, K. Pokrovskaja, K. G. Wiman, G. Klein, and N. Ringertz.** 1995. Reversible nucleolar translocation of EBV encoded EBNA-5 and Hsp70 proteins after exposure to heat shock or cell density congestion. J. Gen. Virol. **76:**2423–2432.
29. **Szekely, L., K. Pokrovskaja, W.-Q. Jiang, G. Selivanova, M. Lowbeer, N. Ringertz, K. G. Wiman, and G. Klein.** 1995. Resting B-cells, EBV infected B-blasts and established lymphoblastoid cell lines differ in their Rb, p53 and EBNA-5 expression patterns. Oncogene **10:**1869–1874.
30. **Szekely, L., G. Selivanova, K. P. Magnusson, G. Klein, and K. G. Wiman.** 1993. EBNA-5, an Epstein-Barr virus-encoded nuclear antigen, binds to the retinoblastoma and p53 proteins. Proc. Natl. Acad. Sci. USA **90:**5455–5459.
31. **Thiry, M.** 1995. Nucleic acid compartmentalization within the cell nucleus by in situ transferase-immunogold techniques. Microscopy Res. Tech. **31:**4–21.
32. **Tomkinson, B., E. Robertson, and E. Kieff.** 1993. Epstein-Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation. J. Virol. **67:**2014–2025.
33. **Weis, K., S. Rambaud, C. Lavau, J. Jansen, T. Carvalho, M. Carmo-Fonseca, A. Lamond, and A. Dejean.** 1994. Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. Cell **76:**345–356.

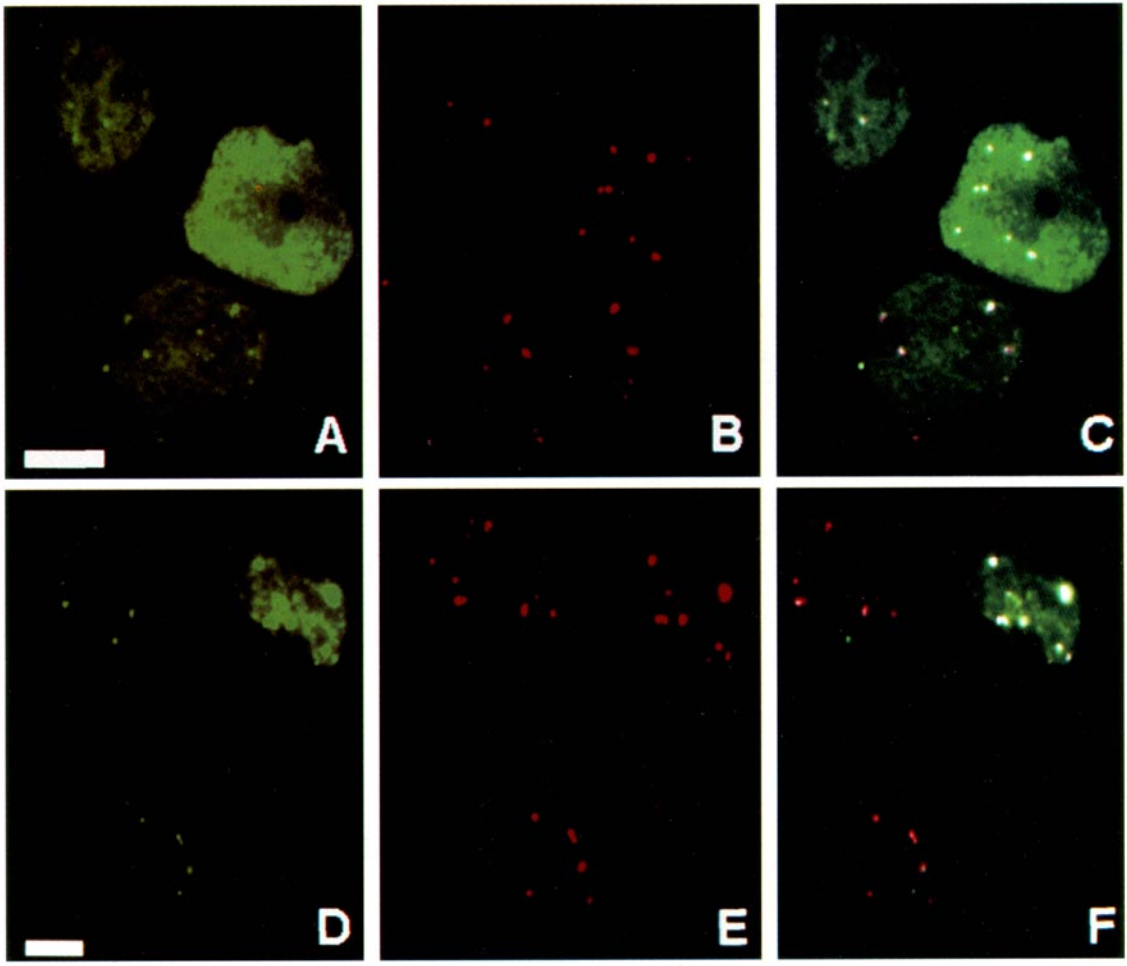

EBNA-5
PML
overlap


A
B
C
D
E
F


A
B
C
D
E
F
G
H
I


A
B
C
D
E


A
B
C
D
E
F


A
B
C
D
E
F