

Association of hepatitis B virus polymerase with promyelocytic leukemia nuclear bodies mediated by the S100 family protein p11

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Abstract

Hepatitis B virus (HBV) polymerase (Pol) interacts with cellular chaperone proteins and thereby performs multiple functions necessary for viral replication. Yeast two-hybrid analysis was applied to identify additional cellular targets required for HBV Pol function. HBV Pol interacted with S100A10 (p11), a Ca²⁺-modulated protein previously shown to bind to annexin II. The interaction between HBV Pol and p11 was confirmed by co-immunoprecipitation of the two proteins synthesized either in vitro or in transfected cells and by inhibition of the DNA polymerase activity of HBV Pol by p11. Immunofluorescence analysis of transfected human cell lines revealed that, although most HBV Pol and p11 was restricted to the cytoplasm, a small proportion of each protein colocalized as nuclear speckles; HBV Pol was not detected in the nucleus in the absence of p11. The HBV Pol–p11 nuclear speckles coincided with nuclear bodies containing the promyelocytic leukemia protein PML. Furthermore, the association of HBV Pol–p11 with PML was increased by exposure of cells to EGTA and inhibited by valinomycin. These results suggest a role for p11 in modulation of HBV Pol function and implicate PML nuclear bodies and intracellular Ca²⁺ in viral replication.

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HBV Pol plays a pivotal role in the viral replication process. Its multiple functions include serving as a signal for the encapsidation of viral pgRNA, priming of reverse transcription, catalysis of both RNA- and DNA-dependent DNA synthesis, and degradation of pgRNA [1]. These multiple functions require the association of HBV Pol with several host factors, including heat shock protein 90 (Hsp90) and its chaperone partner p23 [2], Hsp70 [3–5], and Hsp60 [6].

PML nuclear bodies (PML NBs) are thought to contribute to various cellular functions, such as proliferation, differentiation, survival, and antiviral responses [7], through the actions of the cellular components associated with them. The functions of PML NBs are affected by

pathological conditions such as viral infection and neoplastic transformation. Disruption of PML NBs was seen early during infection with various DNA or RNA viruses [8]. Moreover, treatment of cells with interferon results both in induction of expression of the PML gene and an increase in the number of PML NBs [9]. These observations suggest that PML NBs play a role in the antiviral defense mechanism activated by interferons during viral infection [10]. On the other hand, the replication and transcription of the genome of certain DNA viruses occur near PML NBs, suggesting that these structures are nuclear sites of viral replication [11]. Up-regulation of PML expression has also been detected in tissue affected by inflammation and is associated with inflammatory disorders such as hepatitis [12].

To investigate further the functions of HBV Pol, we have attempted to identify cellular proteins that

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associate with HBV Pol with the use of yeast two-hybrid screening. We now show that HBV Pol interacts with the Ca^{2+} -dependent regulatory protein p11. Our immunofluorescence analysis demonstrates that p11 recruits HBV Pol to PML NBs and that this association is affected by intracellular Ca^{2+} . The interaction between HBV Pol and p11 in PML NBs may provide a new insight into the replication of HBV.

Materials and methods

Yeast and bacterial strains. *Saccharomyces cerevisiae* strains AH109 (*MATa*, *trp1-901*, *leu2-3*, *112*, *ura3-52*, *his3-200*, *gal4 Δ* , *gal80 Δ* , *LYS2::GAL1_{UAS}-GAL1_{TATA}-HIS3*, *GAL2_{UAS}-GAL2_{TATA}-AD E2URA3::MEL1_{UAS}-MEL1_{TATA}-lacZ MEL 1*) and Y187 (*MAT α* , *ura3-52*, *his3-200*, *ade2-101*, *trp1-901*, *leu2-3* *112*, *gal4 Δ* , *gal80 Δ* , *met⁻*, *URA3::GAL1_{UAS}-GAL1_{TATA}-lacZ MEL1*) (Clontech) were used for all yeast two-hybrid analyses. All plasmid manipulations were performed according to standard protocols in the *Escherichia coli* strain DH5 α and all plasmids were introduced into yeast cells by lithium acetate-mediated transformation [13].

Cell lines and antibodies. The cell lines used in this study were obtained from American Type Culture Collection. Mouse monoclonal or rabbit polyclonal antibodies to Myc were from Roche. Mouse monoclonal or rabbit polyclonal antibodies to Flag or to HA, mouse monoclonal antibodies to PML, and rhodamine- or fluorescein isothiocyanate-conjugated goat antibodies to rabbit or mouse were from Santa Cruz Biotechnology.

Yeast two-hybrid analysis. Yeast two-hybrid analysis was performed with the MATCHMAKER 2 hybrid system (Clontech). A human liver-specific cDNA expression library (cloned in pACT2) in Y187 was obtained from Clontech. Complementary DNAs encoding full-length HBV Pol (ayw subtype) or the eight truncation mutants thereof were generated by the polymerase chain reaction and cloned in-frame into the bait vector pGBKT7 (Clontech). The expression of all bait constructs was confirmed by immunoblot analysis with mouse monoclonal antibodies to Myc. Proteins were extracted from transformed yeast cells essentially as described previously [14]. Yeast cells containing a bait vector were mated with the haploid cells harboring the human liver cDNA library, and resulting diploid cells were screened for protein–protein interaction.

GST pull-down assay of HBV Pol and p11 in vitro. Briefly, GST or GST fusion proteins were expressed in DH5 α cells and purified using glutathione–Sephacrose 4B MicroSpin column (Amersham Bioscience). Equal amounts of GST and GST fusion proteins were added into glutathione–Sephacrose 4B MicroSpin columns and incubated for 3 h at 4°C. Five microliters of in vitro translated L-[³⁵S]methionine labeled HBV Pol was then added into each column and incubated at 4°C for 3 h. Bound proteins were eluted, and 20 μ l of each eluted protein was resolved on a 12% SDS–PAGE gel followed by autoradiography.

Co-immunoprecipitation of HBV Pol and p11 in vitro. In vitro transcription and translation were performed with the TNT-coupled reticulocyte lysate system (Promega). The human p11 cDNA was cloned into the yeast AD-vector pGADT7 (Clontech) and subjected to *in vitro* transcription with T7 RNA polymerase. Both HA-tagged p11 and Myc epitope-tagged HBV Pol were translated in vitro in the presence of L-[³⁵S]methionine (Amersham Pharmacia Biotech). Equal portions (50 μ l) of each translation mixture were combined and incubated for 1 h at room temperature before the addition of 1 μ g mouse monoclonal antibodies to Myc or to HA and further incubation for 2 h at 4°C with gentle agitation. Immune complexes were precipitated by the addition of protein G- and protein A-conjugated agarose beads (Oncogene) and, after washing, were either analyzed by SDS–PAGE

(15% gel) and autoradiography or assayed for DNA polymerase activity.

Expression of HBV Pol and p11 in mammalian cells. The coding sequences of p11 and HBV Pol were amplified by the polymerase chain reaction and cloned into the cytomegalovirus promoter-based expression vectors pCMV3B and pCMV2A (Clontech) for expression as Myc epitope- or Flag-tagged proteins, respectively. HepG2, Huh-7, and HeLa cells were grown at 37°C under a humidified atmosphere of 95% air and 5% CO_2 on chamber slides in Dulbecco's modified Eagle's medium supplemented with 0.1 mM nonessential amino acids and 10% fetal bovine serum. The cells were transfected with the use of Effectene (Qiagen).

Assay of HBV Pol activity. The DNA polymerase activity of HBV Pol was assayed as described previously [15]. Transfected cells expressing Flag-tagged HBV Pol were rinsed once with phosphate-buffered saline (PBS) and then lysed with IP buffer [500 mM Tris–HCl (pH 7.5), 1 mM MgCl_2 , 10 mM CaCl_2 , 150 mM NaCl, and 0.1% Triton X-100] supplemented with a mixture of protease inhibitors [1 mM phenylmethylsulfonyl fluoride, aprotinin (4 μ g/ml), leupeptin (0.5 μ g/ml), pepstatin (1 μ g/ml), TPCK (0.5 μ g/ml), and 20 μ M E64]. Cell lysates (1 mg protein) were then subjected to immunoprecipitation with 1 μ g mouse monoclonal antibodies to Flag for 2 h at 4°C with gentle agitation. Immune complexes were precipitated by the addition of 20 μ l protein G- and protein A-conjugated agarose beads, washed five times with IP buffer, and assayed for polymerase activity.

Immunofluorescence analysis. Cells were incubated with 0.5 mM EGTA, 3 μ M cyclosporin A, or 50 μ M BAPTA-AM (Sigma) for 48 h or with 30 μ M valinomycin (Sigma) for 24 h after transfection, as indicated. They were then rinsed with PBS, fixed in 100% methanol for 15 min at –20°C, rinsed twice with PBS, and incubated in blocking solution (1% goat serum in PBS) for 30 min at room temperature. After washing, the cells were incubated overnight at 4°C with rabbit antibodies to Myc (1:200 dilution) for p11, mouse antibodies to Flag (1:200) for HBV Pol, or mouse antibodies to PML (1:200). The cells were then washed before incubation for 1 h at 37°C with appropriate goat secondary antibodies labeled with rhodamine or fluorescein isothiocyanate. Coverslips were applied to the slides in mounting medium containing 4',6-diamidino-2-phenylindole (Vectashield, Vector Laboratories) and the cells were examined either with a Zeiss Axiophot microscope or Nikon inverted microscope equipped with a Hamamatsu Orca charge-coupled device camera, or with a confocal laser scanning microscope (CLSM Bio-Rad 1024). Images were processed with Adobe Photoshop 6.0 software.

Results

Interaction of HBV Pol with p11 in the yeast two-hybrid system

To identify cellular proteins that interact with HBV Pol, we screened a human liver-specific cDNA expression library with the full-length HBV Pol molecule (ayw subtype) as bait [16]. Among a total of $\sim 1.3 \times 10^5$ yeast colonies screened, 17 colonies were selected as positive for all three markers (*HIS3*, *ADE2*, and *lacZ*); the concentration of 3-AT (3-amino-1,2,4-triazole) in the medium was increased to 20 mM in order to reduce the number of false positives. The positive clones included those for Hsp90, which has previously been shown to bind HBV Pol [17], for prostaglandin D_2 synthase (These sequence data have been submitted GenBank database under Accession No. AAH05939), and for the S100

protein S100A10 (p11) as well as four clones encoding proteins of unknown function. In the present study, we focused on the interaction of HBV Pol with p11.

The S100 family has been implicated in the regulation of protein phosphorylation, the activity of various enzymes and transcription factors, the dynamics of cytoskeletal components, cell proliferation and differentiation, and Ca^{2+} homeostasis [18]. These regulatory roles of S100 family members appear to be mediated through the interaction of these proteins with various cellular targets. Thus, p11, a member of this protein family, inhibits annexin II and targets it to cell membranes in mammalian cells [19]. Moreover, p11 interacts with and inhibits the activity of cytosolic phospholipase A_2 [20]; this interaction is mediated by the carboxyl-terminal region of the enzyme and the inhibitory effect is independent of the presence or absence of annexin II. These observations suggest that p11 might function as a chaperone that regulates, through specific interactions, the function or localization of target proteins. We therefore characterized further the interaction of HBV Pol with p11 to determine whether p11 affects the activity or localization of HBV Pol.

We first examined full-length and eight truncated HBV Pol constructs (Fig. 1A) for their potential interaction with p11 in the yeast two-hybrid assay. The interaction between p95 and Mre11 [21] was also assessed as a positive control. Whereas the full-length HBV Pol molecule interacted with p11 in this system, none of the HBV Pol truncation mutants was found to do so (Fig. 1B), indicating that the structural integrity of HBV Pol is required for binding to p11. The specificity of this interaction was verified further by the observation that neither HBV Pol nor p11 interacted with the control proteins Mre11 and p95 (data not shown).

Inhibition of HBV Pol activity by association with p11 in vitro

The interaction of HBV Pol with p11 was first tested in vitro using a GST pull-down assay. The in vitro translated [^{35}S]-labeled HBV Pol could specifically bind to GST-p11 fusion protein but not to GST alone (Fig. 2A). To confirm the direct interaction between HBV Pol and p11, we synthesized Myc epitope-tagged Pol and hemagglutinin epitope (HA)-tagged p11 by in vitro translation in the presence of [^{35}S]methionine. One-half of each translation reaction mixture was then subjected to immunoprecipitation with antibodies to Myc or to HA, and the precipitated proteins were examined by SDS-PAGE and autoradiography; Myc-Pol and HA-p11 were detected at the positions expected for 98- and 12-kDa proteins, respectively (Fig. 2B). The remaining halves of each translation mixture were combined before immunoprecipitation with antibodies to Myc or to HA.

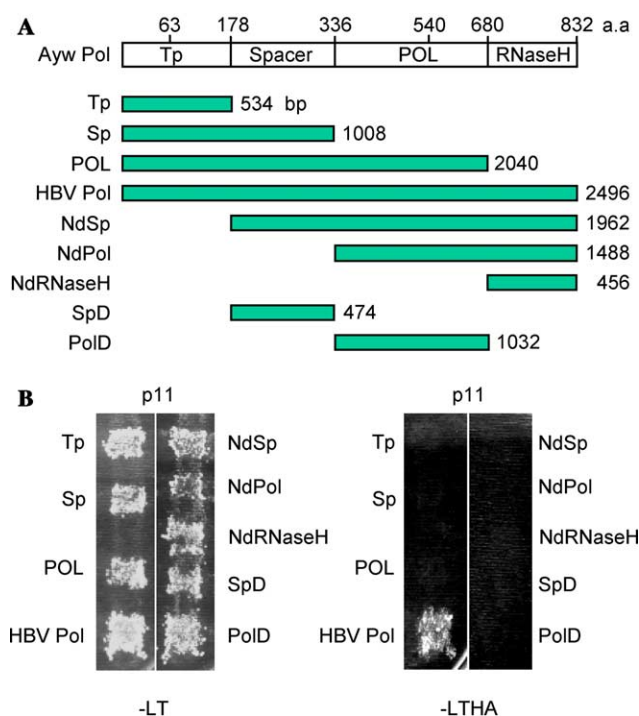


Fig. 1. Interaction of HBV Pol and p11 in the yeast two-hybrid system. (A) Domain organization of HBV Pol and the structures of cDNAs encoding the full-length molecule and eight truncation constructs thereof. (B) Yeast AH109 cells transformed with the bait vector pGBKT7 encoding full-length HBV Pol or one of the eight truncation mutants were mated with Y187 cells expressing full-length p11, and diploid cells were allowed to grow on rich (YPD) medium. The colonies on the mating plates were transferred by replica-plating to SC-Leu-Trp (-LT) plates, on which only diploid cells grow, and were subsequently tested for growth on SC-Leu-Trp-His-Ade (-LTHA) medium.

HBV Pol was coprecipitated with p11 by the antibodies to HA, and, conversely, p11 was coprecipitated with HBV Pol by the antibodies to Myc (Fig. 2B). These results were thus indicative of a direct interaction between HBV Pol and p11 in vitro.

To determine whether its association with p11 affects the function of HBV Pol, we measured the DNA-dependent DNA polymerase (DDDP) activity of the immunoprecipitated HBV Pol-p11 complex with a partially double-stranded, 3'-recessive, synthetic DNA of nonspecific sequence as a template for the filling-in type reaction. Although similar amounts of HBV Pol protein were immunoprecipitated by the antibodies to Myc in the absence or presence of p11, the enzymatic activity of the HBV Pol-p11 precipitate was only ~30% of that of the HBV Pol precipitate (Fig. 2B), likely as a result of the direct inhibition of the catalytic function of HBV Pol by p11. We also examined the interaction between HBV Pol and p11 in cultured mammalian cells. Expression vectors containing the cytomegalovirus early promoter and the coding sequences for Flag epitope-tagged Pol or Myc epitope-tagged p11 were

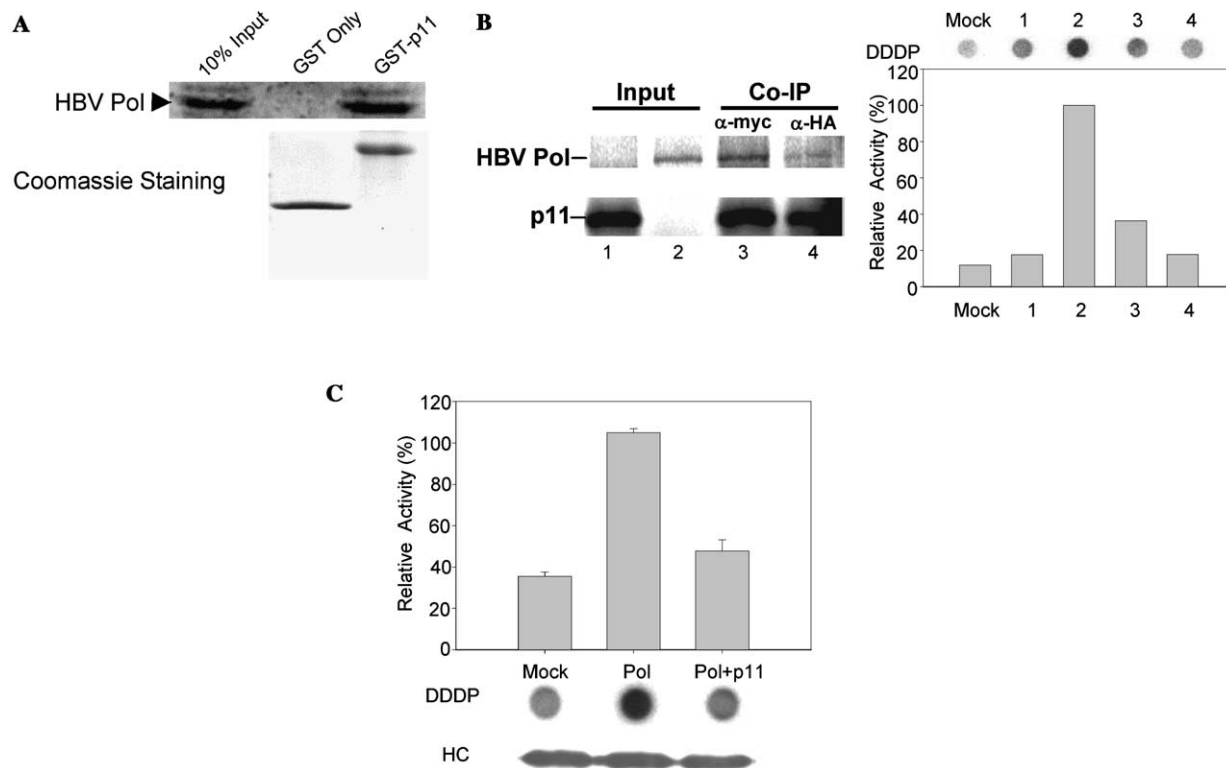


Fig. 2. HBV Pol interacts with GST-p11 in vitro (A) and inhibition of HBV Pol activity through interaction with p11 in vitro (B) and in mammalian cells (C). (A) Analysis of the interaction between HBV Pol and p11 by GST pull-down assay. In vitro translation reaction mixtures containing [³⁵S]methionine-labeled Myc-HBV Pol (~98 kDa) were incubated with GST-p11 or GST-coated beads. Eluted samples and 10% input of [³⁵S]methionine-labeled protein were resolved on a SDS-PAGE gel followed by autoradiography. (B) In vitro translation reaction mixtures containing [³⁵S]methionine-labeled HA-p11 (~12 kDa) and Myc-Pol (~98 kDa) were subjected to immunoprecipitation with antibodies to HA or to Myc, respectively (lanes 1 and 2). Equal amounts of the two reaction mixtures were also combined before immunoprecipitation with antibodies to Myc or to HA (lanes 3 and 4, respectively). The precipitated proteins were both analyzed by SDS-PAGE and autoradiography (left panel) and assayed for DNA polymerase activity (right panel). The polymerase reaction product (DNA-dependent DNA polymerase (DDDP) activity) as well as polymerase activity expressed relative to that of the Pol precipitate in lane 2 are shown. Mock indicates a polymerase reaction performed with proteins was immunoprecipitated with antibody to Myc from a translation mixture containing pGBKT7. (C) 293T cells were transiently transfected for 48 h with an expression vector for Flag-Pol in the absence or presence of a vector for Myc-p11. Cell lysates were then subjected to immunoprecipitation with antibodies to Flag and assayed for DNA polymerase activity. Mock refers to cells transfected with the empty Pol expression vector (pCMV2A). The immunoglobulin heavy chain (HC) of the antibodies to Flag, as detected by SDS-PAGE analysis of the immunoprecipitates, served as an internal control.

introduced into 293T cells by transient transfection. Because of the low level of expression and the instability of HBV Pol in this cell line, we failed to detect the association of HBV Pol with p11 by co-immunoprecipitation analysis. Instead, we demonstrated the inhibition of HBV Pol activity by p11 in the transfected cells (Fig. 2C).

Colocalization of HBV Pol and p11 in mammalian cells

To examine the subcellular localization of HBV Pol and p11 and thereby assess further the interaction between these proteins in intact cells, we performed immunofluorescence staining of cells transfected with expression vectors for Flag-tagged Pol and Myc epitope-tagged p11. We used human hepatoma HepG2 cells for these experiments because they have been

shown to contain little or no endogenous p11 [22]. Immunofluorescence staining of cells expressing Flag-Pol with antibodies to Flag revealed that most of the ectopic HBV Pol was localized in the cytoplasm, with little or none present in the nucleus (Fig. 3). This result is consistent with previous studies of HBV [23] and duck HBV [24] polymerases. In addition, a green fluorescent protein-HBV Pol fusion construct was also restricted to the cytoplasm of HepG2 cells (data not shown). In cells cotransfected with both HBV Pol and p11 vectors, although most HBV Pol and p11 immunoreactivity was colocalized in the cytoplasm, the two proteins were also detected as speckles in the nucleus (Fig. 3). This colocalization of the two proteins in intact human cells is thus consistent with our evidence of their interaction both in vitro as well as in yeast and mammalian cells.

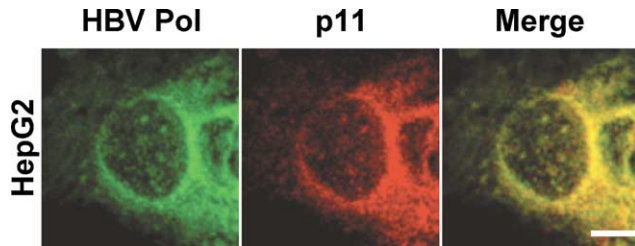


Fig. 3. Colocalization of HBV Pol and p11 in mammalian cells. HepG2 cells cotransfected with expression vectors for Flag-tagged Pol and Myc epitope-tagged p11 were subjected to immunofluorescence analysis with antibodies to Flag (left panel) or to Myc (middle panel). The two images are shown merged in the right panel. The bar represents 10 μ m.

Colocalization of HBV Pol and p11 with PML NBs

The nuclear speckles containing HBV Pol and p11 resembled PML NBs [25]. To examine the possible relation between these structures, we stained HepG2 cells expressing HBV Pol and p11 with antibodies to PML. Most of the nuclear speckles containing HBV Pol were also recognized by the antibodies to PML (Fig. 4A). Given that little or no HBV Pol was detected in the nucleus in the absence of ectopic p11 (Fig. 4B), this colocalization of HBV Pol and PML NBs appears to be dependent on p11. This conclusion is supported by the observation that colocalization of p11 and PML NBs was also apparent in cells expressing recombinant p11 in the absence of HBV Pol (Fig. 4C).

To examine the interaction of p11 and HBV Pol in the context of viral replication, we transfected 2,2,1.5 HepG2 cells, which contain an integrated HBV genome and manifest viral replication and expression of viral proteins, with an expression vector for p11. The association of p11 with PML NBs appeared more marked in this cell line (Fig. 5) than in HepG2 cells. The PML NBs in 2,2,1.5 HepG2 cells expressing recombinant p11 also appeared to be increased in both number and size compared with those in nontransfected cells, suggesting that HBV Pol might affect the structure of PML NBs as a result of its association with p11. The presence of HBV Pol in the nucleus of transfected HepG2 cells was thus not likely due to the lack of viral core particles (and the consequent non-encapsidation of HBV Pol). We also detected the association of HBV Pol and p11 with PML NBs in other cell lines, including Huh-7 hepatoma and HeLa cells (data not shown).

Inhibitory effect of Ca²⁺ on the association of HBV Pol with PML NBs

Given that the activities of the p11 target proteins annexin II and phospholipase A₂ are regulated by Ca²⁺, we investigated whether agents that affect the cytosolic Ca²⁺ concentration modulate the interaction between p11 and HBV Pol in transfected HepG2 cells. Exposure

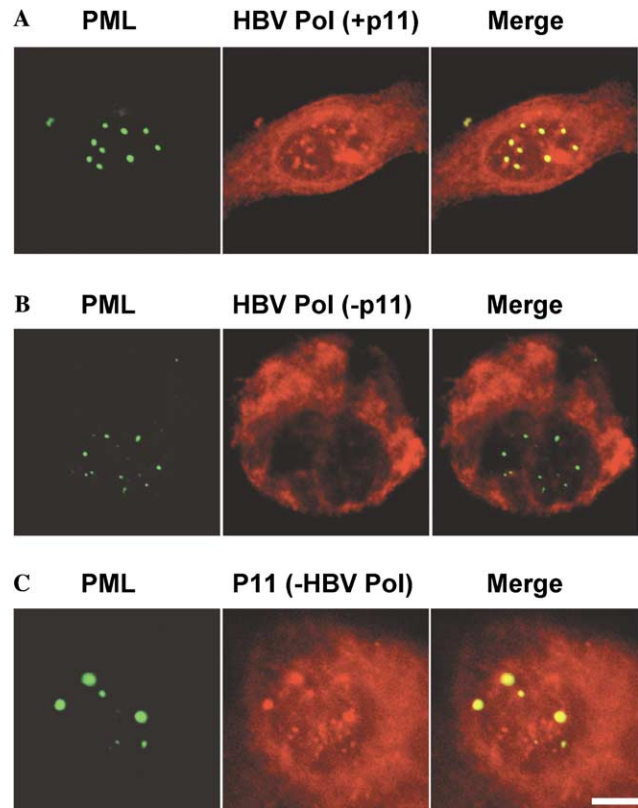


Fig. 4. Colocalization of HBV Pol with PML NBs dependent on p11. HepG2 cells were transfected with expression vectors for both Flag-tagged Pol and Myc epitope-tagged p11 (A), for Flag-Pol alone (B), or for Myc-p11 alone (C). The cells were then subjected to immunofluorescence analysis with antibodies to PML (left panel) and antibodies either to Flag (A and B, middle panel) or to Myc (C, middle panel). The left and middle images are shown merged in the right panels. The bar represents 10 μ m.

of transfected cells to the Ca²⁺ chelator EGTA in order to block the influx of extracellular Ca²⁺ [26] resulted in a marked increase in the number of HBV Pol–p11 speckles in the nucleus (Fig. 6). In contrast, treatment of cells with valinomycin, which promotes Ca²⁺ influx, resulted in a decrease in the amount of HBV Pol and p11 in the nucleus (Fig. 5). We also examined the possible effects of the cell-permeable Ca²⁺ chelator BAPTA-AM

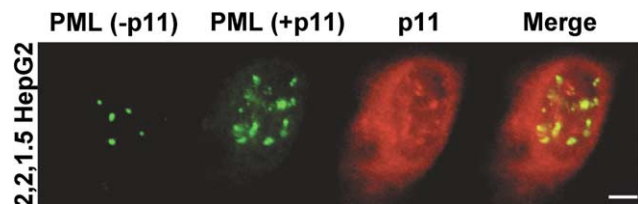


Fig. 5. Association of p11 with PML NBs in 2,2,1.5 HepG2 cells. Cells transfected with an expression vector for Myc epitope-tagged p11 (middle two panels) or not (left panel) were subjected to immunofluorescence staining with antibodies to PML (left two panels) or to Myc (middle right panel). The central two images are also shown superimposed in the right panel. The bar represents 10 μ m.

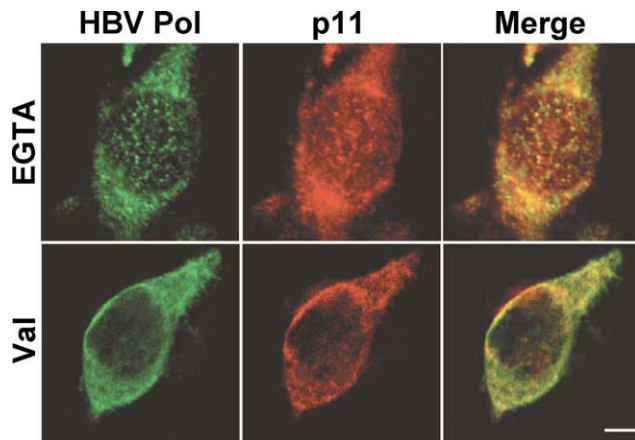


Fig. 6. Effect of Ca^{2+} on the nuclear localization of HBV Pol and p11 in HepG2 cells. Cells transfected with expression vectors for Flag-tagged Pol and Myc epitope-tagged p11 were incubated in the presence of 0.5 mM EGTA for 48 h (upper panels) or with 30 μM valinomycin (Val) for 24 h (lower panels). They were then subjected to immunofluorescence staining with antibodies to Flag (left panels) or to Myc (middle panels); the two images are shown superimposed in the right panels. The bar represents 10 μm .

and of cyclosporin A, on the nuclear localization of HBV Pol and p11 but did not detect any such effect of these agents (data not shown).

Discussion

We have shown that p11 specifically interacts with HBV Pol both in yeast and cultured human cells as well as *in vitro*. Our immunofluorescence analysis also demonstrated that p11 mediates the association of HBV Pol with PML NBs and that this interaction is affected by the cytosolic Ca^{2+} concentration. Moreover, p11 appears to inhibit directly the activity of HBV Pol both *in vitro* and *in vivo*. Although the physiological significance of the interaction between HBV Pol and p11 in PML NBs remains to be determined, our results suggest an active role for p11 in modulation of HBV Pol function and implication of intracellular Ca^{2+} in HBV replication.

Early studies described difficulty in detecting HBV Pol in cultured HepG2 cells [27]. More recent data indicate that this difficulty is due to a low level of HBV Pol expression rather than to inaccessibility of the protein to antibodies as a result of its encapsidation into core particles [28]. In the latter study as well as in other studies in which detectable level of HBV Pol was achieved in Huh-7 cells [29], HBV Pol was shown to be localized exclusively to the cytoplasm, regardless of the presence of other HBV proteins. These observations are consistent with the proposed model of the viral life cycle. Given that reverse transcription takes place inside the nascently assembled viral core particles, it has been

thought that there is no need for HBV Pol to exist outside of the viral capsid.

However, other experimental data appear inconsistent with this model. Thus, a substantial proportion of HBV Pol molecules was detected in the nuclei of liver specimens from individuals with chronic hepatitis [30]. Furthermore, the viral polymerase was also apparent in the nucleus of cells transfected with duck HBV DNA as well as in the nucleus of the virus-infected duck liver [31], even though most duck HBV Pol was present in the cytoplasm in a nonencapsidated form. Finally, Kann et al. [32] showed that the HBV Pol–DNA complex of woodchuck HBV was efficiently transported into the nuclei of digitonin-permeabilized Huh-7 cells when the cellular cytosol was substituted by rabbit reticulocyte lysate and an ATP-generating system, whereas deproteinized viral DNA remained completely outside of the nucleus. These data suggest that HBV Pol might facilitate transport of the viral genome from maturing core particles located in the cytoplasm into the nucleus, thereby contributing to intracellular amplification of viral covalently closed circular DNA.

In addition to this putative role in amplification of viral DNA, HBV Pol is also thought to participate in the transport of incoming viral core particles into the nucleus. After removal of the viral envelope, the viral core is targeted to the nuclear membrane and binds to the nuclear pore complex (through association with importin α and β) in a phosphorylation-dependent manner [33]. The DNA–HBV Pol complex is subsequently released and transported into the nucleoplasm by an unknown mechanism. The intracellular localization of HBV Pol is likely to be determined by cellular factors with which it associates. Our demonstration that HBV Pol localizes to the nucleus in a manner dependent on p11 stresses the importance of a more detailed characterization of the subcellular localization of HBV Pol and its significance in the viral life cycle.

PML has been implicated in the infection of cells by various viruses [34]. Overexpression of PML resulted in an increased resistance of cells to influenza virus and to vesicular stomatitis virus, as manifested by a reduction in virus production [35]. In addition, PML-deficient animals were shown to be 10–100 times more susceptible to lymphocytic choriomeningitis virus and to vesicular stomatitis virus [36]. These observations, together with the induction of PML expression by interferon [37], indicate that PML plays an important role in cellular antiviral defense strategies. PML NBs may thus represent nuclear sites for the accumulation of excess viral proteins and may function as nuclear depots for the elimination of these proteins. Our present data therefore suggest that p11 may function to transport HBV Pol to PML NBs and thereby to inhibit viral replication. An apparently contradictory role for PML NBs is to serve as nuclear sites for the transcription and replication of

the genome of certain DNA viruses, including herpes simplex virus and simian virus 40. Replicating viral DNA has thus been localized near PML NBs by in situ hybridization analysis [38], and transcription of human cytomegalovirus DNA and directional movement of viral transcripts at PML NBs have been described [39]. According to this hypothesis, association of HBV Pol and p11 with PML NBs may help the viral genome transport to the nucleus for initiation of transcription.

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