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# Expression of Recombinant HBV Pol Proteins in HepG2 Cells

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**In this study HepG2 cells were used to express and purify HBV pol proteins. In order to facilitate purification of HBV pol proteins, HBV pol and its deletion mutants were fused to MBP (Maltose Binding Protein). As a result we successfully expressed and partially purified both wild type and mutant recombinant HBV pol proteins by using an amylose resin and anti-MBP antibody. In the case of wild type, the anti-MBP antibody detected three bands. One was full-length and the others were generated by proteolysis of the terminal domain region. The expressed MBP/POL proteins were localized both in the cytoplasm and in the perinuclear region. The purified proteins had polymerase activity toward an exogenous homo-polymer template. The MBP/POL protein also had DNA synthesis activity *in vivo*, since the MBP/POL expression construct was able to complement a HBV polymerase mutant in *trans*.**

**Keywords:** HBVpol, MBP, Deletion mutants, Localization

## Introduction

Human hepatitis B virus is a member of the hepadnavirus family, which are partially double-stranded DNA viruses. The HBV genome is composed of four ORFs: C (core), S (surface), pol (polymerase) and X (transcriptional activator) (Ganem *et al.*, 1987; Ganem, 1996). HBV pol is a multi-functional enzyme that has four domains starting from amino terminus, TP (terminal protein) domain, Spacer, RT (reverse transcriptase) domain, and RNaseH domain (Bartenschlager and Schaller, 1988; zu Putlitz *et al.*, 1999). The TP domain has a tyrosine residue that becomes covalently linked to the minus strand DNA by virtue of the protein-primed initiation of reverse transcription. The RT domain has RNA and DNA dependent elongation activities and the RNaseH domain functions to cleave the RNA strand of an RNA/DNA hybrid. These three activities of HBV pol cooperate to convert the single stranded RNA pregenome found in core particles into double stranded DNA (Ganem and Varmus, 1987; Seeger and Maragos, 1989; Loeb *et al.*, 1991).

The mechanism of HBV pol action, which plays a central role in HBV replication, has been defined poorly due to the difficulty of expressing the active HBV pol. Recently, several studies on the function of HBV pol in genome replication were reported (Wang and Seeger, 1992; Wang and Seeger, 1993; Lanford *et al.*, 1995; Hu and Seeger, 1996; Hu *et al.*, 1997; Lanford *et al.*, 1997; Lanford *et al.*, 1999). In the case of duck HBV, it was demonstrated that the functional pol, obtained from an *in vitro* translation system, has a protein-priming activity (Wang and Seeger, 1993) and an epsilon RNA binding activity (Wang and Seeger, 1992). Their functions also depend on host factors including HSP90, HSP70 and p23, the chaperon partner of HSP90 (Hu and Seeger, 1996; Hu *et al.*, 1997). However, in the case of human HBV a functional pol capable of protein priming was obtained from the baculovirus-insect cell expression system (Lanford *et al.*, 1995; Lanford *et al.*, 1997; Lanford *et al.*, 1999). Schaller *et al.* reported that human HBV pol proteins, expressed by infection of HepG2 cell with a recombinant vaccinia virus, have very short half-lives, very low expression levels, and no polymerase activities as detected by measurements of radioactivity incorporated into a DNA substrate (Bartenschlager and Schaller, 1992).

The recombinant human HBV pol protein that is derived from the baculovirus-insect cell expression system may be different from the recombinant HBV pol that is expressed in mammalian cells. For example, it may particularly lack cellular factors that interact with the HBV pol. In this study we used MBP (Maltose Binding Protein), which is a relatively large sized globular protein (43 KD) as a fusion partner for easy purification of human HBV Pol (Stofko-Hahn *et al.*, 1992). The MBP fusion protein was easily detected in a crude extract by Western blot analysis using an anti-MBP antibody. Conversely, CBP/POL or FLAG/POL that contained shorter fusion partners than MBP were not detected. These facts indicate that the MBP fusion increased expression levels. Also, the expression and purification of several mutants was

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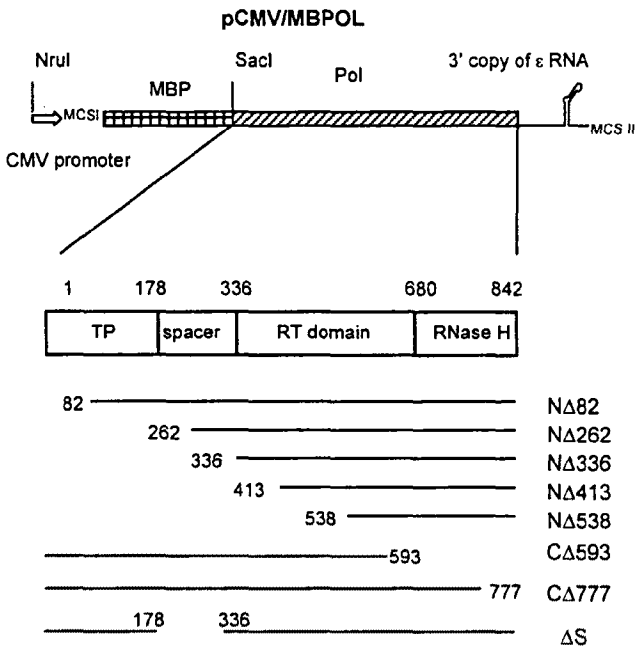


**Fig. 1.** Schematic representation of the expression cassettes, pCMV/MBPOL (7431bp). MCS I contains HindIII, KpnI, BamHI, XbaI, SalI and PstI sites. MCS II contains SalI, PstI and HindIII sites. The plasmid includes the self-transcription terminator used in pregenomic RNA production at the end of the pol coding region. The position of the *pol* gene deletion mutants is indicated. These mutants were cloned into pCMV/MBPOL through the substitution of wild type pol.

successfully accomplished. Their MBP derivatives will be useful for the identification of viral and cellular cofactors that interact with HBV pol protein.

## Materials and Methods

**Plasmid construction** The constructs used for the expression of HBV polymerase are shown in Fig. 1. We introduced a PstI site at the beginning of the coding region of MBP/POL that is derived from pMPH (Jeong *et al.*, 1996) by site-directed mutagenesis using primer (5'-AACAAGGACCATAGATTCTGCAGATGAA AACTGAAGAAGG-3') (Kunkel, 1985). The pUC/MBPOL construct was made by ligating the 4.2 kb PstI fragment containing MBP/POL into a PstI cleaved pUC19. The pCMV/MBPOL was made by the insertion of a ScaI-KpnI fragment of the cytomegalovirus promoter that was derived from Rc/CMV (Akrigg *et al.*, 1985) at the upstream of the MBP/POL coding region (Fig. 1). In deletion mutants, the coding region of all mutants derived from plasmids, pMPH (Jeong *et al.*, 1996; Kim *et al.*, 1999) including the truncated mutant of pol, was replaced with the wild type coding region of pCMV/MBPOL. The pCMV/MBP was constructed by the insertion of a stop codon at the end of the MBP-coding region from the pCMV/MBPOL

**Cell culture and transfection** The human hepatoblastoma HepG2 cells that were used for transfection of the pol expression constructs were cultured on a 100 mm dish in Minimum Essential Medium (MEM) that was supplemented with 10% Fetal Bovine Serum (Life Technologies Inc.). DNA transfection was performed by a liposome method using FuGENE 6 (Roche Molecular Biochemicals) with a total of 15 μg DNA according to the protocol supplied by the manufacturer. HepG2 cells were plated on a 100 mm dish one day before the transfection. The cells were 60% confluent on the day of the transfection. 200 μl of serum free MEM was mixed with 20 μl of FuGENE6. After 5 minutes this solution was added to a tube containing DNA and gently tapped. After incubation for 15 minutes at room temperature a FuGENE6: DNA solution was added to the cells. After 2 days the transfected cells were used for the purification and Western blot analysis of the HBV pol.

**Western blotting analysis and protein purification** Transfected HepG2 cells were harvested and resuspended with 1 ml PBS and protease inhibitor cocktail tablets (Roche Molecular Biochemicals). After freezing and thawing, about 50 micrograms of crude extract were analyzed by 10% SDS-PAGE followed by transfer onto a PVDF membrane and Western blot analysis.

For Western blot analysis we used an anti-MBP polyclonal antibody (1 : 20,000 dilution in PBS containing 1% BSA and 0.05% Tween20), which was made in a rabbit by three injections of MBP antigen purified on an amylose resin. An anti-rabbit polyclonal antibody conjugated with horseradish peroxidase (Sigma Chemical Co. St. Louis, USA) was used as a secondary antibody. Immunoreactive proteins were detected by the ECL Western blotting system (Amersham Pharmacia Biotech Inc.).

To purify the MBP derivatives through an amylose resin, the transfected cells were harvested and lysed with the addition of a 1 ml column buffer (25 mM TrisHCl [pH8.0], 200 mM NaCl, 1 mM EDTA) supplemented with 0.5% NP40 and protease inhibitor cocktail tablets (Roche Molecular Biochemicals). After centrifugation (15,000 X rpm, 15 min) the supernatant was mixed with the activated amylose resin. MBP fusion protein-bound amylose resins were washed four times with a column buffer containing 0.5% NP40. MBP fusion proteins were eluted with the column buffer containing 10 mM maltose. The eluted proteins were used for Western blot analysis.

To immunoprecipitate the MBP/POL, transfected cells (twenty 100 mm dishes; $1.76 \times 10^8$ cells) were harvested and resuspended in 4 ml RIPA buffer (25 mM TrisHCl [pH8.0], 150 mM NaCl, 1 mM EDTA, 1% NP40, 0.5% Triton X-100) containing a protease inhibitor cocktail (Roche Molecular Biochemicals). After centrifugation (14,000 X rpm, 5 min) the supernatant was mixed with 3 μl of an anti-MBP monoclonal antibody (HAM-19; 6 μg) (Park *et al.*, 1998) and 50 μl of protein A-sepharose CL-4B (Amersham Pharmacia Biotech Inc.) for 2 hrs at 4°C. Subsequently, the immunobeads were washed 4 times with a RIPA buffer. Proteins were eluted with 0.1 M glycine [pH 3.0] (60 μl) and neutralized with 0.1 M TrisHCl [pH8.3] (4 μl). Various amounts of the elutants (25 μl, 15 μl and 5 μl) were used for silver staining, immunoblot analysis, and enzyme assay, respectively.

**Subcellular fractionation** Transfected HepG2 cells (two 100 mm dishes) were resuspended in 1 ml of a hypotonic buffer (20

mM TrisHCl [pH7.5], 20 mM NaCl, 2 mM EDTA) that contained a protease inhibitor cocktail and homogenized on ice with 30 strokes of a tight-fitting Dounce homogenizer. Small aliquots of the lysate were taken and stained with trypan blue to determine the progression of cell lysis. Homogenization was continued until >95% of the cells were broken. One ml of lysate was layered onto a 0.6 M to 2 M sucrose gradient and centrifuged with a P55ST2 rotor on a himac HP100α (Hitachi) at 45,000 rpm for 100 minutes at 4°C. After centrifugation, 10 fractions of 500 μl were collected from the bottom of the tube and 20 μl of each fraction was subjected to SDS-PAGE. The MBP/POL proteins were detected by immunoblot analysis.

**Enzyme assays** DNA-dependent polymerase activity assays were carried out as described previously (Jeong *et al.*, 1996). DNA-dependent DNA polymerase activities were monitored by the synthesis of DNA using poly (dA) · oligo $(dT)_{12\text{-}18}$ as template-primer (Amersham Pharmacia Biotech Inc.), respectively. The standard enzyme reaction (50 ml) contained 50 mM TrisHCl [pH 7.4], 50 mM KCl, 0.5 mM $MnCl_2$, 1 mM dithiothreitol, 0.01% NP-40, 50 ng of homo-polymer template (poly (dA) · ligo $(dT)_{12\text{-}18}$), and 2 mCi of [a-$^{32}$P]dTTP (3000 Ci/mmol). Reactions were started by the addition of the immunopurified MBP-HBV polymerase from the transfected HepG2 cells and were incubated at 37°C for 1 hr. Reactions were stopped by the addition of 1 μl of 0.5 M EDTA, followed by spotting on a Whatman DE81 filter paper. Filters were washed four times with 0.5 M $Na_2HPO_4$ [pH 7.0], twice with $dH_2O$, and once with 95% ethanol. Incorporation of radioactivity was determined by liquid scintillation counting in a Packard Tri-Carb Series 1500 liquid scintillation counter.

**Cytoimmunochemistry** For immunofluorescence assays the cells were seeded in four-well chamber slides and transiently transfected with a full-length MBP/POL by the FuGENE6 liposome method (Roche Molecular Biochemicals). After 48 h the cells were fixed with 100% methanol, permeabilized with 0.5% Triton X-100, and sequentially incubated with the primary anti-MBP monoclonal antibodies (HAM-19) (Park *et al.*, 1998) supplied by Dr. Kil-Lyong Kim of KIST (Korea Institute of Science and Technology), followed by anti-mouse immunoglobulin (Ig) covalently coupled to fluorescein isothiocyanate (FITC). Nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI). Then the slide was mounted and observed with an UV filter. The antibody-decorated proteins were visualized directly with a fluorescein isothiocyanate (FITC) filter.

**Preparation of core particles, endogenous polymerase activity assay** Polyclonal antibody against the human HBV core antigen was obtained from DAKO Corp. For the preparation of the core, transfected cells were lysed in 1 ml of TNE (10 mM TrisHCl [pH8.0], 150 mM NaCl, 1 mM EDTA, 1% NP40) as described by Radziwill *et al.* with a minor modification (Radziwill and Schaller, 1990). The lysates were clarified by centrifugation and supernatants were immunoprecipitated with an anti-core antibody and protein A-sepharose L-4B (Amersham Pharmacia Biotech Inc.) for 2 hr at 4°C. Thereafter, the immunobeads were washed 4 times with a TNE buffer. To remove the contaminated nucleic acid, the beads were treated with micrococcal nuclease (Roche Molecular Biochemicals) in the presence of 2.5 mM $CaCl_2$.

For the endogenous polymerase assay, the immunoprecipitate was incubated with dATP, dGTP, dCTP (10 mM each), and 10 μCi of [α-$^{32}$P] TTP (3000 Ci/mmol) (Amersham Pharmacia Biotech Inc.) in a buffer (50 mM TrisHCl [pH8.0], 50 mM $NH_4Cl$, 40 mM $MgCl_2$, 1% NP40 and 0.3% β-mercaptoethanol) for 1 hr at 37°C as described by Radziwill *et al.* (1990). Non-labeled TTP was added to a final concentration of 10 mM for additional incubations (1 hr). Viral nucleic acids, labeled by incorporation of [α-$^{32}$P] TTP, were deproteinized with proteinase K treatment and phenol extraction. The labeled DNA was separated on a 1% agarose gel, transferred to a blotting membrane and analyzed by autoradiography.

## Results

**Transient expression and purification of full-length pol fusion proteins in HepG2 cell** We wanted to take advantage of a fusion protein, such as MBP, in order to overcome the problem of purification of functional pol from HepG2 cells. We transfected HepG2 cells with expression constructs (Fig. 1), which encode full length or truncated human HBV pol with N-terminal MBP fusion (pCMV/MBPOL and deletion mutants). As a result of fusing the 43 kDa MBP to the 90 kDa HBV pol, HBV full-length pol fusion proteins with an apparent molecular weight about 130 kD were easily detected in the crude extract (50 μg) of the transfected cells by using anti-MBP polyclonal antibody in an immunoblot (Fig. 2). When the calmodulin binding peptide

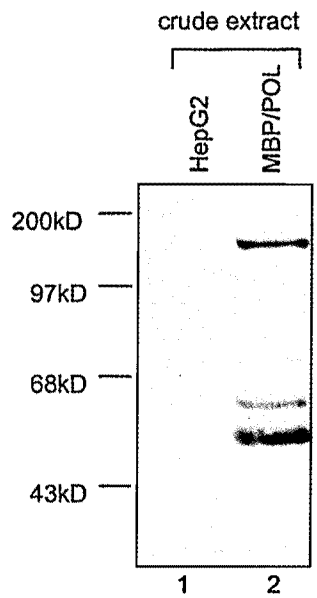


**Fig. 2.** Western blot analysis of the expressed MBP/POL fusion protein. Detection of MBP/POL proteins in the crude extract (50 μg) of HepG2 cells transfected with pCMV/MBPOL by immunoblotting using an anti-MBP antibody. Lane 1, non-transfected HepG2 cells as negative control. Lane 2, pCMV/MBPOL-transfected HepG2 cells. Three bands were detected with an anti-MBP antibody.

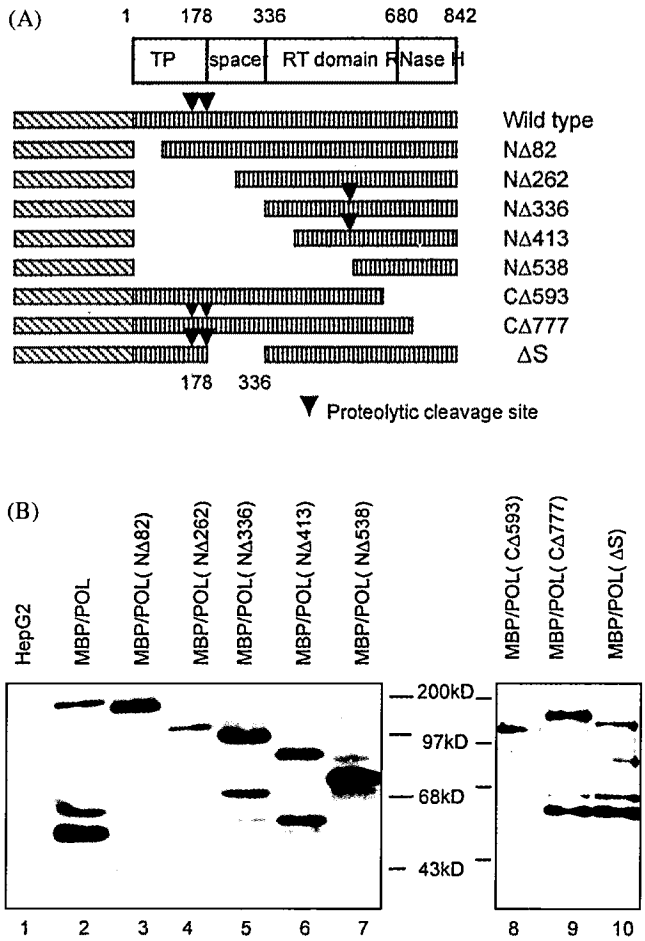


**Fig. 3.** Western blot analysis of MBP/POL and derivatives partially purified using amylose resin. Each band was detected using an anti-MBP antibody as described in *Materials and Methods*. A). Schematic representation of the mutant proteins. B) N-terminal deletion, C-terminal deletion and spacer deletion mutant. Lane 1, non-transfected cell as negative control. Lane 2-10, wild type MBP/POL, NΔ82, NΔ262, NΔ336, NΔ413, NΔ538, CΔ593, CΔ777 and ΔS, respectively.

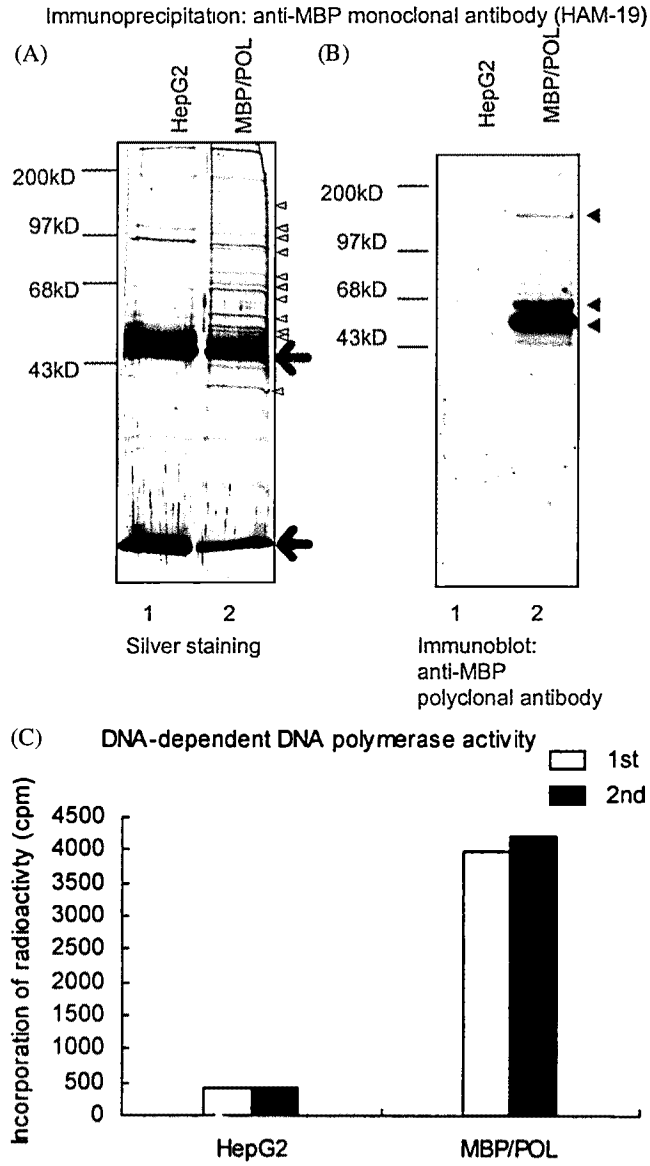


**Fig. 4.** Silver staining (A) and Western blotting (B) of the immunoprecipitated MBP/POL proteins. Lane 1. Immunoprecipitates from the non-transfected HepG2 cells. Lane 2. Immunoprecipitates from the transfected HepG2 cells with pCMV/MBPOL. Blank arrows indicate the specific bands that were immunopurified by an anti-MBP antibody in a silver-staining gel. Arrow is a heavy and light chain of immunoglobulin. Arrowheads indicate the major bands detected by the anti-MBP antibody in immunoblot. C) DNA-dependent DNA polymerase activity. Enzyme assay was performed as described in *Materials and Methods*. The two experiments were performed independently.

(CBP) or FLAG peptide was fused to HBV pol, it was not detected with a biotinylated calmodulin or anti-FLAG antibody (M2) (data not shown). These facts indicated that MBP fusion increased the expression level of HBV pol. The two bands observed, in addition to the full-length MBP/POL which probably result from proteolytic cleavage of the HBV polymerase portion of the fusion proteins, were approximately 64 and 58 kDa as determined by SDS-PAGE. They reacted with an antibody directed toward the MBP amino terminus (Fig. 2). The cleavage sites inferred from molecular weight calculations are probably in the TP (terminal protein) domain (about 145 and 175 amino acid residues, respectively).

Purification of the expressed MBP/POL proteins in HepG2 cells was tried by chromatography using an amylose resin. The fraction eluants from the amylose resin were analyzed by an immunoblot using an anti-MBP polyclonal antibody. As shown on the immunoblot in Fig. 3, three MBP/POL derivatives were successfully purified. This result indicates that the pol portion that was fused to MBP did not prevent the MBP portion from binding to the amylose resin. But there were many protein contaminants on the Coomassie brilliant blue (CBB) stained SDS-PAGE.

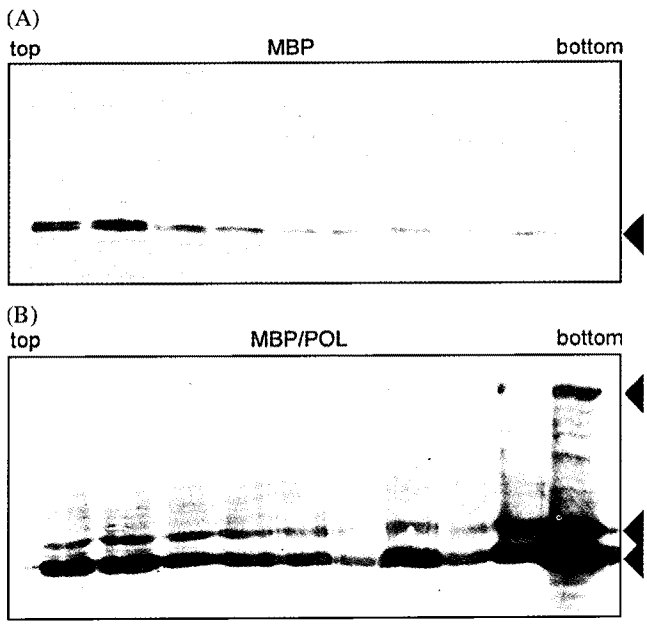


**Fig. 5.** A subcellular fractionation of the transfected HepG2 cells with pCMV/MBPOL and pCMV/MBP. After centrifugation each fraction was analyzed by SDS-PAGE and immunoblot using an anti-MBP antibody. Arrowheads indicate the band detected by the anti-MBP antibody.

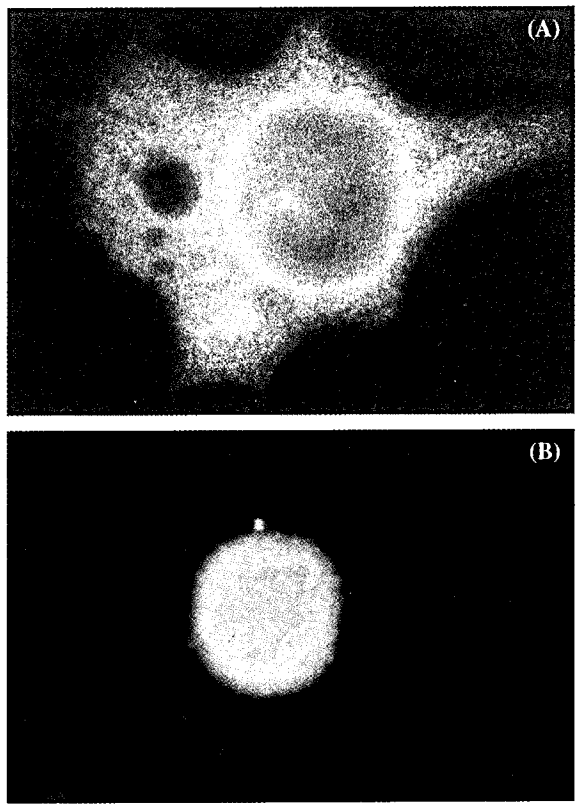


**Fig. 6.** Immunocytochemistry of cells transfected with pCMV/MBPOL. A) Transfected HepG2 cells stained with an anti-MBP monoclonal antibody. B) Transfected HepG2 cells stained with DAPI. The transfection efficiency was about 7% as estimated by counting the cell stained with the anti-MBP antibody. A cytoplasmic signal corresponding to HBV pol was detectable in cells transfected with pCMV/MBPOL. Also, the perinuclear region was densely decorated with an anti-MBP antibody.

In order to obtain the purer MBP/POL proteins, immunoprecipitation with an anti-MBP monoclonal antibody (HAM-19) was performed as described in *Materials and Methods*. The eluants were analyzed by silver staining and immunoblotting using an anti-MBP polyclonal antibody (Fig. 4). Compared to the negative control elutants from non-transfected HepG2 cells, the elutants from pCMV/MBPOL-transfected HepG2 cells comprised the specific bands, as shown on the silver-stained SDS-PAGE (Fig. 4). In the immunoblot analysis, three major MBP/POL derivatives, accompanied by the minor bands, were detected with anti-MBP antibody. There were also protein contaminants. These proteins were not present in the immunoblot. The yield of recombinant full-length proteins was approximately 40 ng per $1.76 \times 10^8$ cells (20 dishes). The amount of the purified MBP/POL protein is smaller than the estimated amount of the expressed MBP/POL proteins in crude extracts (about 1.2 mg per $1.76 \times 10^8$ cells). There is a possibility that most of the expressed MBP/POL proteins may not be localized to the cytoplasm. To investigate this possibility, we performed the subcellular fractionation experiment as described in *Materials and Methods*. After centrifugation, each fraction is analyzed by immunoblotting using an anti-MBP polyclonal antibody (Fig. 5). In the case of MBP used as a negative control, MBP proteins were mostly detected in the vicinity of the top fraction. But, MBP/POL proteins were predominantly detected in the vicinity of the bottom fraction containing nuclei. To confirm the localization of MBP/POL, the intracellular localization by indirect immunofluorescence was studied. Although MBP/POL proteins exist in cytoplasm, it localized predominantly to the perinuclear region, which is the major component of the insoluble fraction (Fig. 6). These results were consistent with Schaller's report that pol expressed in HuH-7 HCC cells is exclusively localized in the cytoplasm and perinuclear region (Bartenschlager and Schaller, 1992). We found that the low yield of purification was derived from the existence of the insoluble MBP/POL in the perinuclear region.

The immuno-purified MBP/POL proteins were used for polymerase activity assay due to a purity of MBP/POL. Polymerase activity of the purified proteins with a supply of the exogenous homo-polymer template (poly (dA)-oligo $(dT)_{12\text{-}18}$) was surveyed (Fig. 4). As a result, it was found that the purified MBP/POL proteins were active for the DNA-dependent DNA polymerase function.

To examine whether or not the expressed MBP/POL protein is active *in vivo*, we performed an endogenous polymerase activity assay (EPA). After a 1.2 HBV genome, DNA was transfected in the HepG2 cell line, the EPA result of

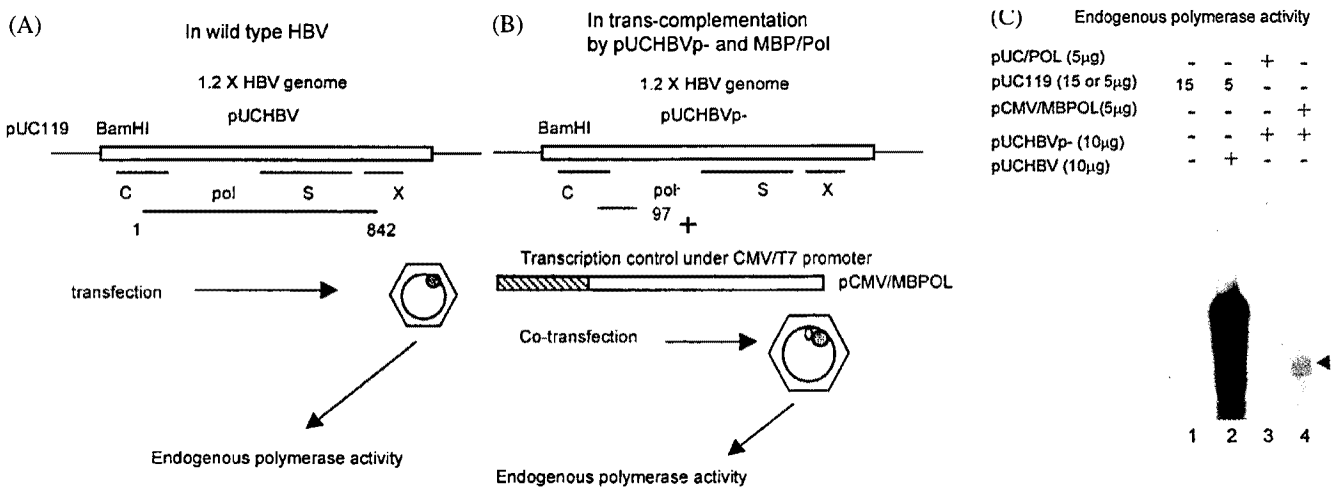


**Fig. 7.** Characterization of recombinant HBV core particles produced by co-transfection of pUCHBV and pUC119 (A) or pUCHBVp-[CΔ97] and pCMV/MBPOL (B) into HepG2 cells. Schematic representation of the generation of recombinant virus. Three days after transfection, intracellular core particles were purified by immunoprecipitation. These core particles were used to assay endogenous polymerase. The purified core particle was incubated with radioactive [α-$^{32}$P] TTP and non-radioactive dATP, dCTP and dGTP for 1 hr. Non-labeled TTP was added to a final concentration of 10 mM for additional incubations (1 hr). After proteinase K treatment, the labeled DNAs were extracted with phenol and electrophoresed on an 1% agarose gel. B) The result of endogenous polymerase activity assay. The kind and quantity of transfected DNA was described on each lane of the figure. Lane 1, non-transfection. Lane 2, co-transfection of pUC119 and pUC/HBV. Lane 3, co-transfection of pUC119 and pUC/HBVp-[CΔ97]. Lane 4, co-transfection of pUC/HBVp-[CΔ97] and pCMV/MBPOL. Arrowheads indicate the short DNA fragment generated by MBP/POL.

the core particle immunopurified from the transfected cell had the polymerase activity against endogenous template (Fig. 7). As shown in Fig. 5B, HepG2 cells were co-transfected with expression constructs coding for the MBP/POL proteins and a 1.2 HBV mutant genome which contains a frame-shift mutation in the *pol* gene (Fig. 7). After the immunopurification of the core particle as described in *Material and Methods*, we tested the endogenous polymerase activity (EPA) of the core particle with a supply of the radioactive nucleoside. An endogenous polymerase activity assay of the core particle, which was generated by trans complementation between the MBP/POL expression construct and the 1.2 HBV mutant genome, labeled the nucleic acid (Fig. 7). But, the quantity of labeled nucleic acid generated by MBP/POL was lower than wild type pol. As a result, it appeared that the MBP/POL expressed in HepG2 cells could replicate their genome by complementation of the HBV mutant genome (Fig. 7).

**Transient expression and purification of mutants pol fusion proteins in HepG2 cell** To obtain mutant proteins, the mutant expression plasmids were transfected, the proteins were partially purified, and Western blot analysis was performed as described in *Materials and Methods*. In C-terminally deleted mutants and the spacer domain deletion mutant (Fig. 3), the purified mutant proteins had slightly increased electrophoretic mobility compared with the full-length MBP/POL protein on the blot. Also, the previously described extra two bands, generated by specific proteolysis of the terminal protein domain portion (about 145 and 175 amino acid residues) of HBV polymerase, appeared in the CΔ777 deleted mutant and the spacer deletion mutant, but not

**Table 1.** Purification and DNA Polymerase Activity of Recombinant MBP/POL Proteins from the transfected HepG2 cells.

| Number of HepG2 cells | Total protein in lysate (mg) | The immunopurified MBP/POL proteins (ng) [1)] | DNA pol activity (cpm)[2)] |
|---|---|---|---|
| $1.76 \times 10^8$ (tranfected) | 12.2 | 20 | 4210 |
| $2.1 \times 10^8$ (non-transfected) | 16.2 | ND | 436 |

[1)]The quantitation of purified MBP/POL proteins is estimated from the intensity comparison of full-length MBP/POL band to control MBP protein on the immunoblot.

[2)]The DNA polymerase activity was monitored by the synthesis of DNA using poly (dA). Oligo $(dT)_{12\text{-}18}$ as template-primer (Amersham-Pharmacia Biotech Inc.).

The used proteins in the DNA polymerase activity assay was one-fourth of the eluted MBP/POL proteins from $1.76 \times 10^8$ HepG2 cells. The non-transfected HepG2 cells were used as a negative control.

in the CΔ598 deleted mutant.

On the other hand, the band patterns of the purified N-terminal deletion mutant proteins (Fig. 3) were different than those of the C-terminal deletion mutants and the spacer deletion mutant. The full-length bands of the N-terminal deletion mutants were exactly positioned on the blot. Only the purified protein pool of NΔ336 and NΔ413 mutants contained other bands generated by proteolysis (68 kDa and 60 kDa, respectively). NΔ82 and NΔ262 mutants did not show the proteolytic products. The cleavage sites of NΔ336 and NΔ413 inferred from the calculation of molecular weight are in the RT domain (about 530 amino acid residues). Here, MBP/POL proteins were expressed and partially purified.

## Discussion

The purpose of this study was to express and purify HBV pol in HepG2 cells. The data suggested that the fusion of MBP to HBV pol increased the expression level in a HepG2 cell. Also, MBP/POL derivatives were easily detected by western blotting using an anti-MBP antibody and partially purified using an amylose resin or anti-MBP antibody. Although the expression levels varied in each transfection experiment due to transfection efficiency, there was about 50 ng MBP/POL protein per 100 mm transfected dish (48 hours after transfection), as estimated from Western blot analysis. But the purified MBP/Pol proteins are very small compared to the expressed MBP/POL proteins in crude extract. This low purification efficiency results from the existence of the expressed MBP/POL proteins in the perinuclear region. In the previous report we demonstrated that MBP/POL fusion proteins expressed in *E. coli* were active for polymerase activity (Jeong *et al.*, 1996; Kim *et al.*, 1999). Also, the purified MBP/POL fusion proteins from HepG2 cells represented the polymerase activity for the exogenous homo-polymer template. This system, compared to pol expression using vaccinia virus expression system, increased the expression level of pol protein and supplied the easy one-step affinity purification for the active pol protein in the HepG2 cell.

The polymerase elongation activity of MBP/POL was detected with an endogenous polymerase assay (EPA) using the core particle generated by complementation of the MBP/POL protein. This result indicates that the MBP/POL protein acted as an active polymerase *in vivo*. Interestingly, the short DNA fragments appeared mostly in the case of MBP/POL in comparison with pUCHBV (Fig. 7). Mutational analysis for HBV pol protein showed that full-length pol proteins are required for HBV replication (Bartenschlager and Schaller, 1988; Hirsch *et al.*, 1990; Radziwill and Schaller, 1990; Pollack and Ganem, 1994). These facts indicate that the degradation products of MBP/POL do not produce the short DNA fragments. Our result was that the generation of the short DNA products were derived from MBP fusion to TP domain of HBV pol (unpublished data).

Recent results obtained with the duck hepatitis B virus (DHBV) indicated that the reverse transcription of HBV pol also depends on host factors that include the heat shock protein Hsp90 (Hu and Seeger, 1996; Hu, J. *et al.*, 1997). Using this expression system we showed that MBP-fused human HBV pol protein interacted with endogenous HSP90 (Cho *et al.*, 2000). This expression system may also provide an invaluable tool for the identification of viral and cellular cofactors that interact with the HBV pol protein.

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