KOREAN JOURNAL OF ZOOLOGY
Vol. 28, No. 3, 166-178 (1985)

Cloning of the Hepatitis B Surface Antigen Containing Pre-surface Antigen Region and Poly(A) Addition Site

Sang Hae Kim, Yong Sok Kim, Mee Young Choi, and Hyune Mo Rho
(Department of Zoology, College of Natural Science, Seoul National University)

Pre-surface antigen 지역과 poly(A) addition site가 포함된 B형 간염 표면항원 유전자의 재조합

김상해 · 김용석 · 최미영 · 노현모
(서울대학교 자연과학대학 동물학과)

(Received May 15, 1985)

적 요

한국형 B형 간염바이러스(HBV) DNA의 표면항원 유전자를 포유동물 세포에서 발현시켜 항원의 검출과 유전자의 분자유전학적인 연구를 하기 위하여 pre-surface antigen 지역과 표면항원 유전자 그리고 poly(A) addition site가 포함된 DNA 조각을 simian virus 40(SV 40)의 DNA 복제 원점과 promoter가 포함된 유전자 운반체에 재조합 시켰다. 우선, HBV의 전체 DNA가 들어있는 pHBV 107을 Bam HI으로 부분절단한뒤 self-ligation시켜 두 HBV DNA가 같은 방향으로 들어간 pHBVD 107을 만들었다. 이 plasmid를 Bgl II로 절단하였을때 pre-surface 지역과 표면항원 유전자 그리고 poly(A) addition site가 함께 포함된 2.7 kb의 insert DNA 조각을 얻었다.

유전자 운반체로는 포유동물세포에서 복제할 수 있도록 하기 위하여 SV40의 DNA 복제 원점부위와 72 bp repeats(enhancer)가 포함된 pSVOE를 만든 다음 이 vector의 Pvu II 절단자리에 Bam HI linker를 붙여 insert DNA가 vector의 SV40 late promoter지역 가까이에 들어갈 수 있도록 변형시킨 pSVOB를 만들었다. 이상과 같이 만들어진 pre-surface 지역-표면항원유전자-poly(A)-addition site가 포함된 2.7 kb DNA 절편을 pSVOB promoter 뒤의 Bam HI site에 삽입하여 재조합된 plasmid pSVBS를 얻었다. 예비실험으로 pSVBS를 T-antigen이 생산되는 COS cell에 이주시켰더니 HBsAg가 발현됨을 보았다.

INTRODUCTION

Hepatitis B virus (HBV) causes serious human liver disease including hepatoma (Robin-

son, 1977; Szmuness, 1975; Vyas *et al.*, 1974). Although intensive efforts have been made to understand the structure and behavioral characteristics of this virus, the biological study of the HBV has been hampered by the inability to propagate in any tissue culture system and its narrow host range (Tiollais *et al.*, 1981). Nevertheless, considerable recent progress has been made in elucidating the structure of the HBV virion and its genome (Galibert *et al.*, 1979; Pasek *et al.*, 1979; Valenzuela *et al.*, 1979). The HBV virion (the Dane particle) is a 42-nm sphere with an outer envelope being the hepatitis B surface antigen (HBsAg) and an inner nucleocapsid being a second antigen, the hepatitis B virus core antigen (HBcAg) (Blumberg *et al.*, 1975; Dane *et al.*, 1970). HBsAg represents both the major envelope protein and the neutralizing antigen of the infectious virion and as such has proven effective as a vaccine against the disease (Purcell and Gerin, 1975; Szmuness *et al.*, 1980). Surface antigen (HBsAg) is composed of several antigenic determinants; the "a" determinant which is group-specific and two pairs of mutually exclusive determinants, d/y and w/r (Dreesman *et al.*, 1972). The electrophoretic analysis of the envelope proteins shows the presence of two to seven polypeptides (Patrick *et al.*, 1979). The two major ones are called; P24 and its glycosylated form, GP27. HBsAg 20-nm particles from viremic donors (Stibbe and Gerlick, 1982) also contain two minor glycoproteins, GP33 and GP36 (Sanchez *et al.*, 1981; Skelly *et al.*, 1978), which have not yet been well characterized. It was necessary to know if these proteins were coded by the viral or the cellular genome. Recently, the cloning and expression of HBsAg in *E. coli* (Burrell *et al.*, 1979; Charnay *et al.*, 1979; Edman *et al.*, 1981; Eujisawa *et al.*, 1983: Mackay *et al.*, 1981) and in yeast (Valenzuela *et al.*, 1982; Miyanohara *et al.*, 1983; Hitzeman *et al.*, 1983; McAleer *et al.*, 1984) and the introduction and expression of cloned HBV DNA in mammalian cells by using simian virus 40 (SV40) based recombinant plasmid have been reported (Moriarty *et al.*, 1981; Crowley *et al.*, 1983; Siddiqui, 1983; Simonsen and Levinson, 1983; Will *et al.*, 1984). Also this chimeric vector, which is nonlytic and thus not constrained by packaging considerations (Mulligan *et al.*, 1979; Gething and Sambrook, 1981), replicate to approximately 10^5 copies per cell and produce HBsAg particles similar to those found in the serum of infected patients (Liu *et al.*, 1982; Crowley *et al.*, 1983). But little is known about the subtype adr HBV which is spread mainly in Far East Asia including Korea (Ono *et al.*, 1983). For this reason we constructed the recombinant plasmid pSVBS, SV40/HBV chimeric plasmid, to characterize HBV in mammalian cells. To facilitate expression of HBsAg in mammalian cells, we constructed the vector pSVOB containing SV40 sequences with the origin of DNA replication and 72bp repeats (enhancer). Such vector is capable of replicating in monkey cells provided large T antigen is present to initiate succesive rounds of DNA synthesis. Expression of the heterologous genes in COS cell was preliminarily observed when they were inserted behind 72 bp repeats of SV40 of the pSVOB.

MATERIALS AND METHODS

Bacterial strain: E. coli strain HB101(F^-, r^-, m^-, recA13) was used as host cell for transformation with plasmid DNA and for purification of plasmids. Cells were grown at 37°C in LB medium (10 g of Bacto trypton, 5 g of Yeast extract and 10 g of NaCl per liter)

DNAs and Enzymes: Plasmid DNAs were extracted by the procedure of Birnboim and Doly (1979) and further purified by CsCl density gradient ultracentrifugation (Clewell and Hellinski, 1969). All restriction endonucleases including T4 DNA ligase were purchased from Bethesda Research Laboratories (BRL) and New England Biolabs. Bam HI linkers were purchased from New England Biolabs.

Enzymatic Reactions: All reactions of restriction enzymes were performed at 37°C in common digestion buffer (33mM Tris-HCl, pH 7.0, 66 mM KAc, 10 mM MgAc, 0.5 mM DTT) for 1 hour. After reaction, samples were incubated at 65°C for 10 minutes to inactivate restriction enzyme activity. The fragments with blunt ends generated by Pvu II digestion and Bam HI linker were ligated at 22°C for 18 hours (Bahl *et al.*, 1976; Scheller *et al.*, 1977). The ligation of DNA fragments with cohesive ends were performed as described by Kahn *et al.* (1983).

Transformation: Procedure was based on the method by Kushner (1978). Grown cells (5×10^7 cells/ml, $OD_{600}=0.6$) were harvested and resuspended in 10 mM MOPS (morpholinopropane sulfonic acid), pH 7.0, 10 mM RbCl. Cells were recovered by centrifugation and resuspended in 0.1 M MOPS, pH 6.5, 50 mM $CaCl_2$ and 10 mM RbCl. After incubation for 15 minutes, cells were recovered and resuspended in one fifth volume of above solution. DNA and DMSO were added and incubated on ice for 30 minutes. The competent cell mixture was mixed with LB media and plated on LB agar plate containing appropriate antibiotics.

Plasmid Screening: To screen the proper plasmid from transformed colonies, 1 ml cultured cells were resuspended in 0.05 M Tris-HCl, pH 6.8, 1% SDS, 2 mM EDTA, 0.4 M sucrose, 0.01% bromophenol blue, and incubated at room temperature for 30 minutes. Gel electrophoresis of DNA was performed on 0.8~0.1% horizontal agarose gel as described by Sharp *et al.*(1973).

RESULTS

Construction of the plasmid pHBVD 107: The schematic diagram of constructing pHBVD 107 containing tandem copies of the HBV genome in a head-to-tail arrangement was shown in Fig. 1. A recombinant plasmid, pHBV 107, was constructed by inserting Bam HI-cleaved DNA from Dane particles into Bam HI site of plasmid pBR 322(Choi *et al.*, 1984). But

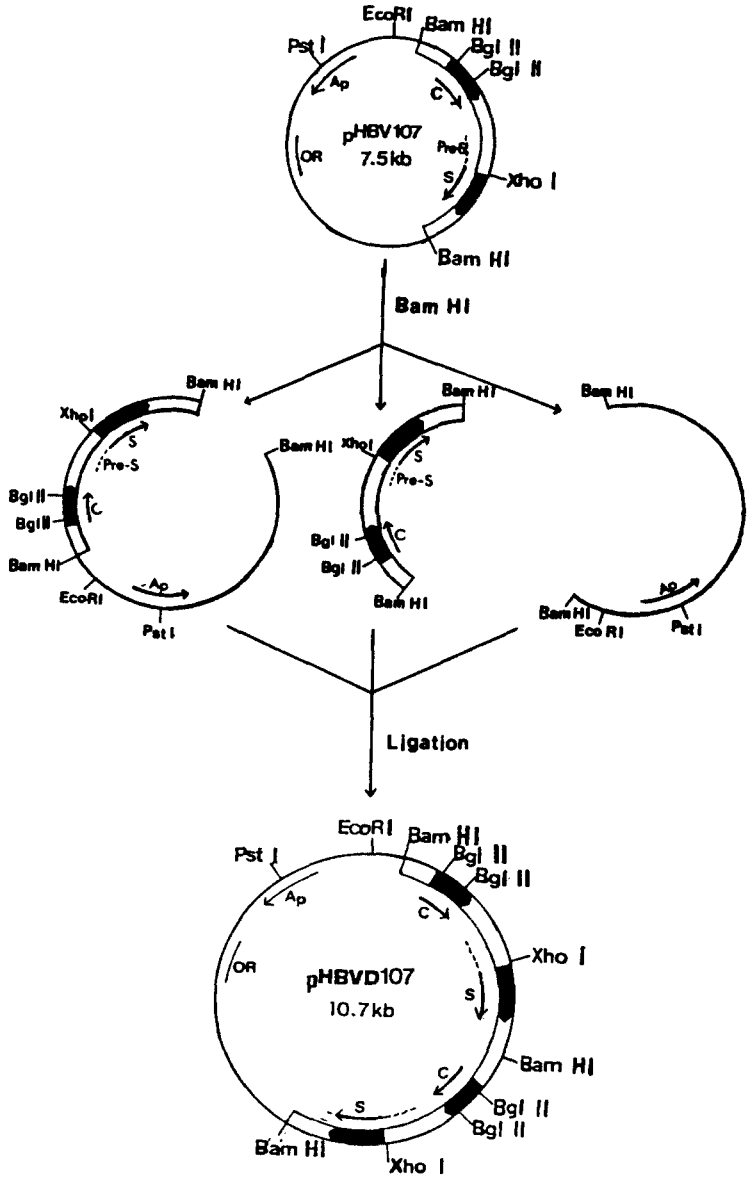

Fig. 1. The strategy for the construction of pHBVD 107. pHBVD 107 was constructed by partial digestion and self-ligation of the pHBV 107 as described previously. The direction of transcription is shown by arrows. The thick line represents the region of HBV DNA and the thin line represents the pBR 322 DNA. Ap: ampicillin, OR: the pBR 322 replication origin, C: core antigen gene of HBV, S: surface antigen gene of HBV, pre-S: pre-surface region of HBV.

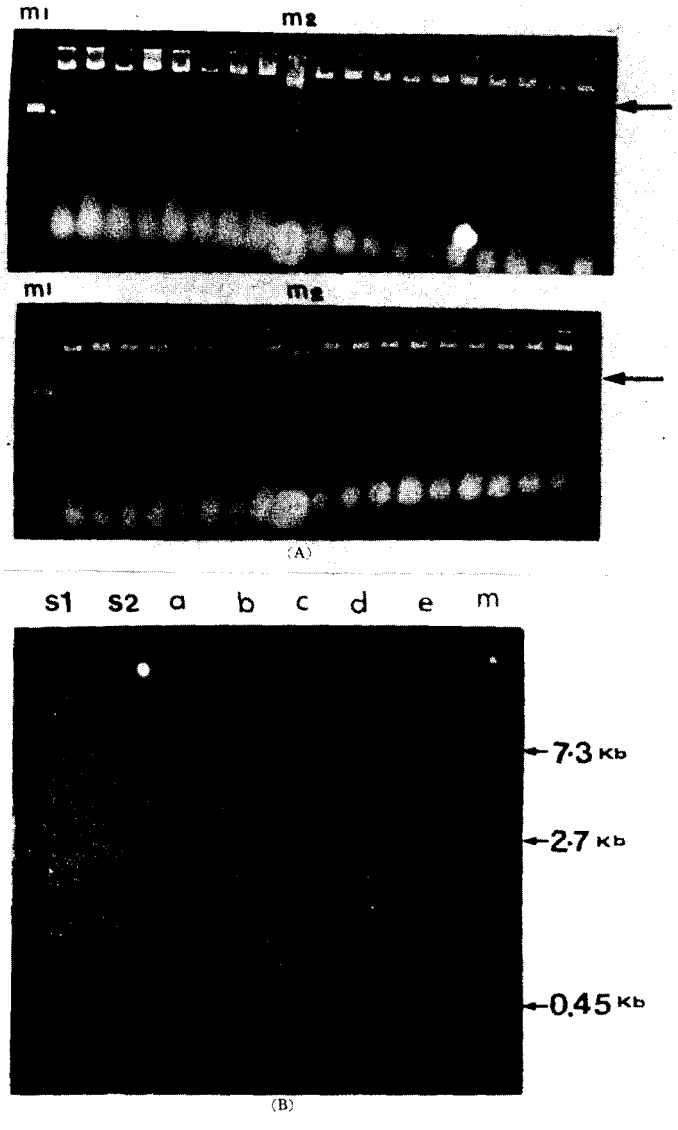

Fig. 2. Screening and restriction endonuclease analysis of the pHBVD 107.

(A) Cracking pattern as a primary screening for pHBVD 107. An arrow indicates the colonies containing larger plasmids than pHBV 107. m_1 indicates plasmid pHBV 107 and m_2 is pBR 322.

(B) Restriction endonuclease analysis of pHBVD 107. The recombinant plasmid was digested with several restriction endonucleases and analyzed on 0.8% agrose gel. Lane S_1, pHBV 107: S_2, pHBVD 107: a, pHBV 107+Bam HI: b, pHBVD 107+Bam HI: c, pHBV 107+Eco RI: d, pHBVD 107+Eco RI: e, pHBVD 107+Bgl II: m, λ DNA+Hind III marker.

it was necessary to construct the plasmid containing head-to-tail dimers of HBV DNA because monomeric genome interruped at the unique Eam HI site between surface antigen gene and poly (A) addition site did not direct HBsAg biosynthesis in mammalian cells. Therefore, pHBVD 107 containing tandem copies of the HBV genome in a head-to-tail

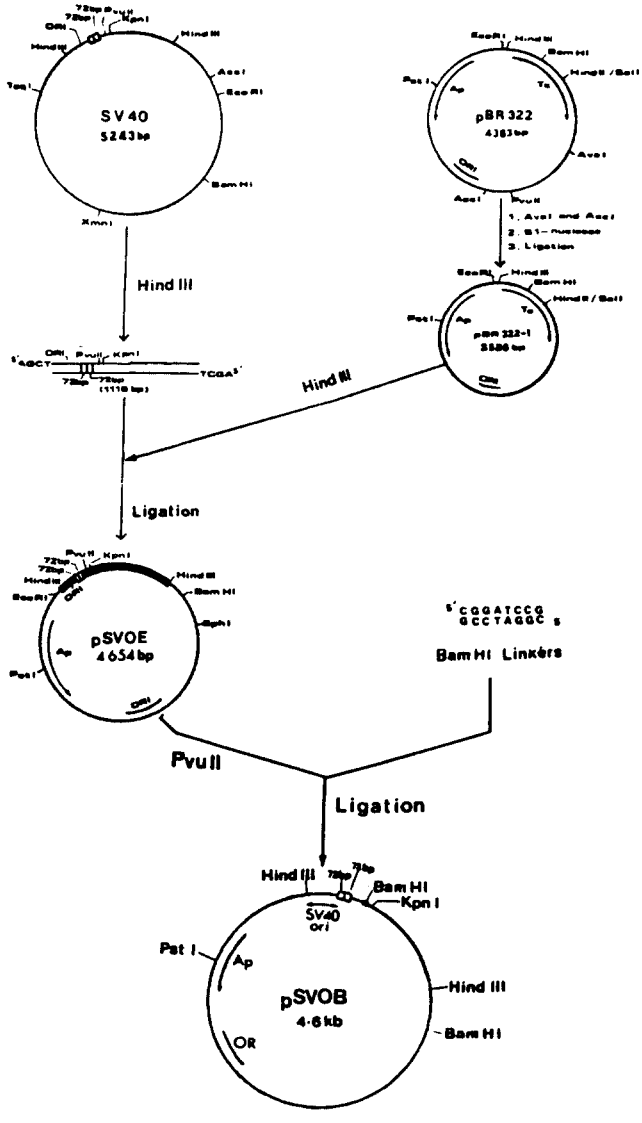

Fig. 3. The strategy for the construction of the vector pSVOE and pSVOB.
The solid box in pSVOE represents 1,118 Hind III fragment of SV 40 and the open box in pSVOB represents 72bp repeats of SV 40. Bam HI linker was phosphoryrated by T4 DNA kinase before it was joind to pSVOE.

arrangement was constructed. pHBV 107 was partially digested with Bam HI, self-ligated, and transformed in *E. coli* HB 101 as described in MATERIALS AND METHODS. Resistant colonies to ampicillin (80 μg/ml), over 500 colonies, were collected. Through the cracking procedure and restriction enzyme analysis, 10.7 kb plasmid which contain tandem copies of the HBV genome in a head-to-tail arrangement was found (Fig. 2). pHBVD 107 cleaved by Eco RI (Fig. 2B, lane d) shows linear form. When pHBVD 107 was digested with Bam HI (lane b), two bands of 4.3 kb and 3.2 kb fragment were detected. It indicates that two 3.2 kb HBV DNA fragment were overlaped. For the determination of the polarity of two HBV genomes, pHBVD 107 was digested with Bgl II (lane e), producing 7.3 kb, 2.7 kb and 0.45 kb DNA bands. Therefore, it strongly suggested that pHBVD 107 should have tandem copies of the HBV genome in a head-to-tail arrangement.

Construction of plasmids pSVOE and pSVOB: We constructed chimeric vectors, pSVOE and pSVOB to express the HB_sAg in mammalian cells. The 1,118 bp Hind III fragment of SV40 contains sequences capable of promoting the synthesis of mRNA in both the early and late directions (Crowley *et al.*, 1983; Moriarty *et al.*, 1981; Tegtmeyer, 1972) and serves as an origin of replication of the plasmid when introduced into mammalian cells expressing SV40 large T antigen (Gluzman, 1981; Mellon *et al.*, 1981). The general approach for the construction of vectors involved the incorporation of SV40 sequences spanning the viral origin of replication into plasmid pBR 322-1, that is a pBR 322 derivative lacking sequences which inhibit replication in monkey cells (Lusky *et al.*, 1981). pBR 322-1 was constructed by Ava I and Acc I codigestion of pBR 322. DNA fragment (3,536 bp) produced by Ava I and Acc I digestion was treated with S1 nuclease to produce blunt ends and ligated. To prepare the vector pSVOE, pBR 322-1 was opened with Hind III and inserted 1,118 bp Hind III fragment of SV40 (Fig. 3). To insert Bgl II-cleaved fragment into the region behind the late promoter of SV40, Bam HI linker

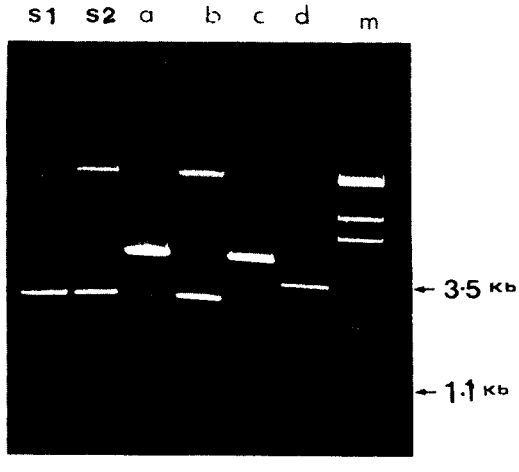

Fig. 4. Restriction endonuclease analysis of pSVOB. pSVOB was digested with several restriction endonuclease and analyzed on 0.8% agarose gel. Lane S_1, pSVOE: S_2, pSVOB: a, pSVOE+Bam HI: b, pSVOB+Pvu II: c, pSVOB+Eco RI: d, pSVOB+Bam HI: m, λ DNA+Hind III marker.

having the same restriction site as Bgl II site was inserted into Pvu II site of pSVOE. pSVOE was digested with Pvu II and ligated with Bam HI linker. pSVOB with an additional Bam HI site was identified by restriction endonuclease analysis (Fig. 4). Lane b and d in Fig 4 showed that Bam HI linker was inserted into Pvu II site of pSVOE.

Construction of the recombinant plasmid pSVBS: Figure 5 outlined the general strategy for the construction of the recombinant plasmid pSVBS. 2.7 kb DNA fragment (Fig. 2B, lane e) containing the sequences for pre-surface region-HB_sAg gene poly(A) addition site derived from the digestion of pHBVD 107 with Bgl II, inserted into the Bam HI site of pSVOB by ligation. Recombinant plasmids were selected by the criteria of the generation of 3.5 kb and 2.7 kb DNA fragments from plasmids by the Bam HI and Eco RI (Fig.

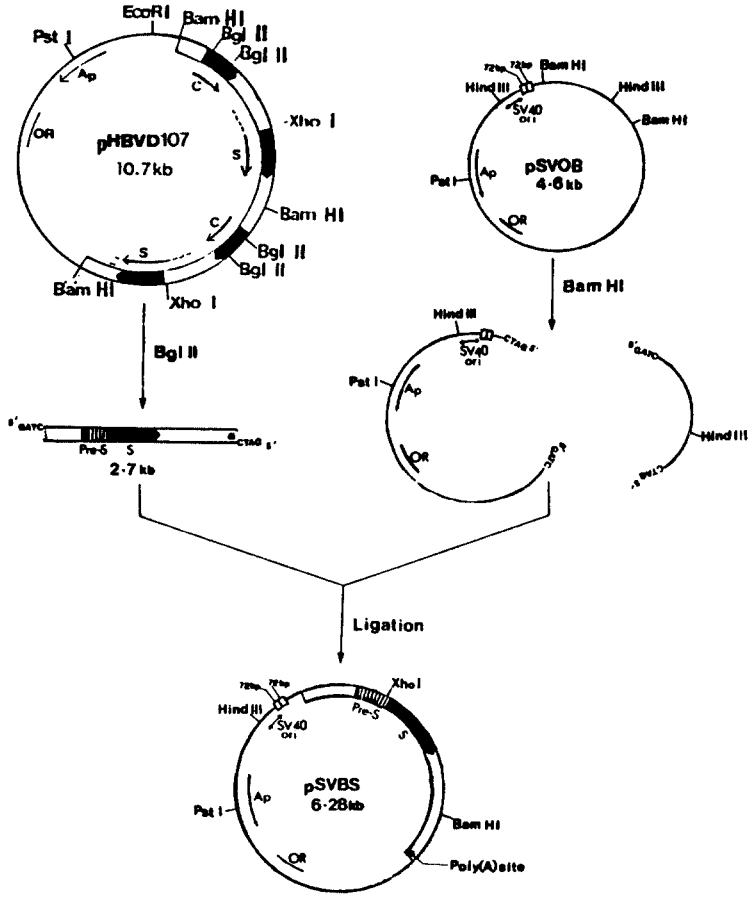

Fig. 5. The strategy for the construction of the recombinant plasmid pSVBS. The surface antigen and the core antigen genes are shown as solid boxes, the pre-s region as hatched box, and the 72 bp repeats of SV 40 as open box.

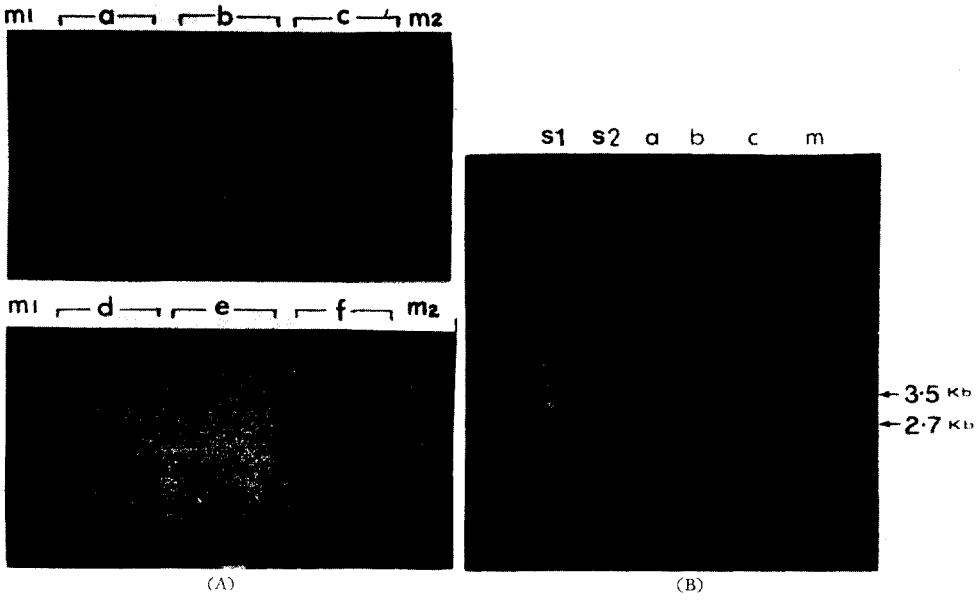

Fig. 6. Restriction endonuclease analysis of pSVBS.
(A) The electrophoresis pattern by Bam HI and Eco RI digestion as a screening of pSVBS. The first lane of each sample (a-f) shows the plasmid of uncut-supercoil form. The middle lane shows the plasmid digested with Bam HI and the right lane shows the plasmid digested with Eco RI. Lane m_1 is pBR 322 and m_2 is λDNA+Hind III marker.
(B) The restriction endonuclease analysis of pSVBS. The recombinant plasmid was digested with selected restriction endonucleases and analyzed on 0.8% agarose gel. Lane S_1, pSVOB: S_2, pSVBS: a, pSVBS+Bam HI: b, pSVBS+Bam HI+Eco RI: c. pSVBS+ Eco RI: m, λDNA+ Hind III marker. Arrows indicate the DNA bands of pSVBS (3.5kb and 2.7kb) generated by co-digestion with Bam HI and Eco RI.

6B, lane b). Insert DNA fragment and late promoter sequences of SV40 had the same transcriptional polarity in this plasmid and named as pSVBS. COS cell producing T antigen was transfected with pSVBS. Preliminary assay showed that the transformed cell produced HB_sAg, which will be published elsewhere.

DISCUSSION

The HB_sAg of HBV gene products has been used as a marker for the HBV infection and demonstrated its structure and function by numerous studies. The HB_sAg contains a group specific determinant (a) and type specific determinant (d/y and w/r). Thus four major subtypes of HB_sAg (adw, ayw, adr, and ayr) denote the phenotypes of the virion (Dreesman *et al.*, 1972). The adr HBV was mainly found in the Southeast Asia and Korea (Yamashita *et al.*, 1975; J.Y. Kim, Personal Commum.) and the study for this subtype was limited. Previously, in our laboratory, the adr HBV genome extracted from

the serum of the infected Korean was cloned in *E. coli* to construct restriction map and for the expression of its HB_sAg gene (Choi *et al.*, 1984). Although many laboratories have also cloned and studied HB_sAg gene in bacteria, they have not answered clearly the questions related to the HB_sAg synthesis and crystalization. For these reasons, it was necessary to clone HB_sAg gene in mammalian cells. Recently, several groups have reported the introduction and expression in mammalian cells of the gene encoding HB_sAg. These efforts have utilized either dorminant selectable markers (Christman *et al.*, 1982; Dubois *et al.*, 1980) or lytic vector based on SV40 (Liu *et al.*, 1980; Moriarty *et al.*, 1981). Advantages of virus-based vectors such as SV40 are that a high vector copy number is assured and a high level of heterologous gene expression can be observed. However, there are several drawbacks, strict packaging constraints and lytic infection. In this study, we constructed the recombinant plasmid pSVBS to express the HB_sAg containing pre-surface region and poly(A) addition site in mammalian cells. pSVBS comprise SV40/pBR 322 chimeric vector pSVOB containing the origin of viral replication, both the early and late promoters, and 72 pb repeats (enhancer). This vector is nonlytic and capable of replicate to 10^5 copies per cell (Crowley *et al.*, 1983; Liu *et al.*, 1982). The recombinant plasmid pSVBS can replicate and express the HB_sAg in mammalian cells constitutively producing T antigen to support the replication of SV40. We used COS cell as a host cell of pSVBS introduction in the preliminary study. COS cell is monkey kidney cell transformed with defective SV40 and thus synthesize T antigen in sufficient quantities. Preliminary assay with AUSRIA II showed that the transformed cells with pSVBS produced HB_sAg, which will be published elsewhere.

We will extensively characterize the HB_sAg produced in this expression system and this sytem should afford the opportunity to study not only factors controlling the expression of a foreign gene in mammalian cells, but also provide a convenient experimental system permitting the study of the process involved in the posttranslational modification, assembly and secretion of complex macromolecular aggregates in such cells. HB_sAg produced in COS cell in the further preliminary study will be subject to characterization about the structure and function of it by the various assay including radioimmunoassay, immunofluorescence, sucrose density gradient centrifugation, and electron-microscope.

SUMMARY

In order to express hepatitis B surface antigen (HB_sAg) containing pre-surface antigen region in mammalian calls, 2.7 kb DNA fragment containing pre-surface region-HB_sAg gene poly(A) addition site of HBV genome was cloned into simian virus 40(SV40) based chimeric vector pSVOB. 2.7kb DNA fragment was derived from pHBVD 107 containing tandem copies of the HBV genome in a head-to-tail arrangement by Bgl II digestion. Construction of the vector pSVOE involved the incorporation of SV40 sequences spanning

the viral origin of replication and 72 bp repeats (enhancer) into a pBR 322 derivative lacking sequences which inhibit replication in mammalian cells. Bam HI linker was inserted at the Pvu II site in the proximity of SV40 late promoter of pSVOE and named as pSVOB. To construct the recombinant plasmid pSVBS, pHBVD 107 was digested with Bgl II to isolate 2.7kb DNA fragment and the fragment was ligated into the Bam HI site of pSVOB by ligation. Preliminary result showed that the recombinant plasmid pSVBS produced HB_sAg in the monkey cell producing large T antigen (COS cell).

REFERENCE

Bahl, C.P., K.J. Manans, R. Wu, J. Slawinsky and S.A. Narang, 1976. A general method for inserting specific DNA sequences. *Gene* **1**:81.

Birnboim, H.C. and J. Doly, 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**:1513-1523.

Blumberg, B.S., B. Larouze, W.T. London, B. Werner, J.E. Hesser, I. Millman, G. Saimot and M. Payet, 1975. The relationship of infection with the hepatitis B antigen to primary hepatic carcinoma. *Am. J. Pathol.* **81**:669-682.

Burrell, C.T., P. Mackay, P.J. Greenaway, P.H. Hofshneider and K. Murray, 1979. Expression in *E. coli* of hepatitis B virus DNA sequences cloned in plasmid pBR 322. *Nature* (*London*), **279**: 43-47.

Charnay, P., C. Pourcel, A. Louise, A. Fritsch and P. Tiollais, 1979. Cloning in *Eschenchia coli* and physical structure of hepatitis B virion DNA. *Proc. Natl. Acad. Sci. U.S.A.* **76**:2222-2226.

Choi, M.Y., Y.S. Kim and H.M. Rho, 1984. Cloning and genetic mapping of the hepatitis B viral genome. *J. Korean Soci. Virology* **14**:49-55.

Christman, J.K., M. Gerber, P.M. Price, C. Flordellis, J. Edelman and G. Acs, 1982. Amplification of expression of hepatitis B surface antigen in 3T3 cells cotransfected with a dominant-acting gene and cloned viral DNA. *Proc. Natl. Acad. Sci. U.S.A.* **79**:1815-1819.

Clewell, D.B. and D.R. Hellinski, 1969. Supercoiled circular DNA protein complex in *Escherichia coli:* Purification and induced coversion to an open circular DNA form. *Proc. Natl. Acad. Sci. U.S.A.* **62**:1159-1166.

Crowley, C.W., C.C. Liu and A.D. Levinson, 1983. Plasmid directed synthesis of hepatitis B surface antigen in monkey cells. *Mol. Cell. Biol.* **3**:44-55.

Dane, D.S., C.H. Cameran and M. Briggs, 1970. Virus-like particles in serum of patients with Australia antigen-positive hepatitis. *Lancet* **1**:695-698.

Dreesman, G.R., R. Chariez, M. Saurez, F.B. Hollinger, R.J. Courtney and J.L. Melnick, 1975. *J. Virol.* **16**:508-515.

Dubois, M.F., C. Pourcel, S. Rousset, C. Chany and P. Tiollais, 1980. Excretion of hepatitis B surface antigen particles from mouse cells transformed with cloned viral DNA. *Proc. Natl. Acad. Sci. U.S.A.* **77**:4549-4552.

Edman, J.C., R.A. Hallewell, P. Valenzuela, H.M. Goodman and W.J. Rutter, 1981. Synthesis of hepatitis B surface antigens in *E. coli. Nature* (*London*) **291**:503-506.

Fujisawa, Y., Y. Ito, R. Sasada, Y. Igarash, R. Marumoto, M. Kikuchi and Y. Sugino, 1983. Direct expression of hepatitis B surface antigen gene in *E. coli*. *Nucleic Acids Res.* **11**:3581-3591.

Galibert, F., E. Mandart, F. Fitoussi, P. Tiollais and P. Charnay, 1979. Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in *E. coli*. *Nature* (*London*) **281**:646-650.

Gething, M.J. and J. Sambrook, 1981. Cell-surface expression of influenza haemagglutinin from a cloned DNA copy of the RNA gene. *Nature* (*London*) **283**:620-625.

Gluzman, Y., 1981. SV40 transformed simian cells support the replication of early SV 40 mutants. *Cell* **23**:175-182.

Hitzeman, R.A., C.Y. Chen, F.E. Hagie, E.J. Patzer, C.C. Liu, D.A. Estell, D.G. Kleid, A.D. Levinson, J.V. Miller, A. Yaffe and H. Oppermann, 1983. Expression of hepatitis B virus surface antigen in yeast. *Nucleic Acids Res.* **11**:2745-2763.

Kahn, M., R. Kolster, G. Thomas, D. Figurski, R. Meyer, E. Remout and D.R. Helinski, 1983. Plasmid cloning vehicles derived from plasmids Col El, F, R6K, and RK2. *Methods Enzymol.* **68**:268.

Kushner, S.R., 1918. An improved method for transformation of *E. coli* with Col El-derived plasmids. In Genetic engineering (ed. H.B. Boyer and S. Nicosia). P. 17 Elsevier/North-Holland, Amsterdam.

Liu, C.C., D. Yansure and A.D. Levinson, 1982. Direct expression of hepatitis B surface antigen in monkey cells from an SV 40 vector. *DNA* **1**:213-221.

Lusky, M. and M. Botchan, 1981. Inhibition of SV40 replication in simian cells by specific pBR 322 DNA sequences. *Nature* (*London*) **293**:79-91.

Mackay, P., M. Pasek, M. Magazin, R.T. Kovacic, B. Allet, S. Stahl, W. Gilbert, H. Schaller, S.A. Bruce and K. Murray, 1981. Production of immunologically active surface antigens of hepatitis B virus by *E. coli*. *Proc. Natl. Sci. U.S.A.* **78**:4510-4514.

McAleer, W.J., E.B. Buynak, R.Z. Maigetter, D.E. Wampler, W.J. Miller and M.R. Hilleman, 1984. Human hepatitis B vaccine from recombinant yeast. *Nature* (*London*) **307**:178-180.

Mellon, P., V. Parker, Y. Gluzman and T. Maniatis, 1981. Identification of DNA sequences required for transcription of the human α1-globin gene in a new SV 40 host vector system. *Cell* **27**:279-288.

Miyanohara, A., A. Toh-E, C. Nazaki, F. Hamada, N. Ohotomo and K. Matsubara, 1983. Expression of hepatitis B surface antigen gene in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **80**:1-5.

Moriarty, A.M., B.H. Hoyer, J.W. Shih, J.L. Gerin and D.H. Hamer, 1981. Expression of hepatitis B virus surface antigen gene in cell culture by using a simian virus 40 vector. *Proc. Natl. Acad. Sci. U.S.A.* **78**:2606-2610.

Mulligan, R.C., B.H. Howard and P. Berg, 1979. Synthesis of rabbit β-globin in cultured monkey kidney cells following infection with a SV 40 β-globin recombinant genome. *Nature* (*London*) **277**:108-114.

Myers, R.M. and R. Tjian, 1980. Construction and analysis of simian virus origins defective in tumor antigen binding and DNA replication. *Proc. Natl. Acad. Sci. U.S.A.* **77**:6491-6495.

Ono, Y., H. Onda, R. Sasada, K. Igarashi, Y. Sugino and K. Nishioka, 1983. The complete nucleotide sequences of the cloned hepatitis B virus DNA subtype adr and adw. *Nucleic Acids Res.* **11**:1747-1757.

Pasek, M., T. Golo, W. Gilbert, B. Zink, H. Schaller, P. Mackay, G. Lead better and K. Murray,

1979. Hepatitis B virus genes and their expression in *E. coli*. *Nature* (*London*) **282**:575-579.

Purcell, R.H., Gerin and J.B. Almeida, 1973/74. Radioimmunoassay for the detection of the core of the Dane particle and antibody to it. *Intervirology* **2**:231-243.

Robinson, W.S., 1977. The genome of hepatitis B virus. *Ann. Rev. Microbiol.* **31**:357-377.

Sanchez, Y., I. Ionescu-Matiu, G.R. Dreesman, F.B. Hollinger and J.L. Melnick, 1981. Evidence for the presence of repeating antigenic determinants in the major polypeptides derived from hepatitis B surface antigen. *Virology* **114**:71-80.

Scheller, R.H., R.E. Dickerson, H.W. Boyer, A.D. Riggs and K. Itakus, 1977. Chemical synthesis of restriction enzyme recognition sites useful for cloning. *Science* **196**:177.

Sharp, P.A., B. Sugden and J. Sambrook, 1973. Detection of two restriction endonuclease activities in *Haemophilus parainfluenza* using analylical agarose. *Biochemistry* **12**:3055.

Siddiqui, A. 1983. Expression of hepatitis B surface antigen gene in cultured cells by using recombinant plasmid vectors. *Mol. Cell. Biol.* **3**:143-146.

Simonsen, C.C. and A.D. Levinson, 1983. Analysis of processing and polyadenylation signals of the hepatitis B virus surface antigen gene by using simian virus 40-hepatitis B virus chimeric plasmids. *Mol. Cell. Biol.* **3**:2250-2258.

Skelly, J.C., C.R. Howard and A.J. Zuckerman, 1978. The labelling of galactose residues in hepatitis B surface antigen glycoprotein. *J. Gen. Virol.* **41**:447-457.

Stibbe, W. and W.H. Gerlick, 1982. Variable protein composition of hepatitis B surface antigen from different donors. *Virology* **123**:436-442.

Szmuness, W. 1978. Hepatocellular carcinoma and the hepatitis B virus, evidence for a causal association. *Prog. Med. Virol.* **24**:40-69.

Szmuness, W., C.E. Stevens, E.J. Harley, E.A. Zang, W.R. Oleszak, D.C. William, R. Sadovsky, J.M. Morrisor and A. Kellner, 1980. Hepatitis B vaccine. Demonstration of efficiency in a controlled clinical trial in a high risk population in the United States. *New Engl. J. Med.* **303**:833-841.

Tegtmeyer, P. 1972. Simian virus 40 deoxyribonucleic acid synthesis: the viral replicon. *J. Virol.* **10**:591-598.

Tiollais, P., P. Charnay and G.N. Vyas, 1981. Biology of hepatitis B virus. *Science* **213**:406-411.

Valenzuela, P., P. Gray, M. Quiroga, J. Zaldivar, H.M. Goodman and W.C. Ratter, 1979. Nucleotide sequence of the gene coding for the major protein of hepatitis B virus surface antigen. *Nature* (*London*) **280**:845-819.

Valenzuela, P., A. Medina and W.J. Rutter, 1982. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature* (*London*) **298**:347-350.

Vyas, G.N. and N.R. Shulamn, 1970. Hemagglutination assay for antigen and antibody associated with viral hepatitis. *Science* **170**:332-333.

Will, H., R. Cattaneo, E. Pfaff, C. Kuhn, M. Rogenorf and H. Schaller, 1984. Expression of hepatitis B antigens with a simian virus 40 vector. *J. Virol.* **50**:335-342.

Yamashita, Y., S. Kurashina, Y. Miyakawa and M. Mayumi, 1975. *J. Infect. Dis.* **131**:567-569.