Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7708–7712, December 1984
Biochemistry

Synthesis in animal cells of hepatitis B surface antigen particles carrying a receptor for polymerized human serum albumin

(gene amplification/S1 nuclease mapping/mice immunization/recombinant hepatitis B vaccine/Chinese hamster ovary cells)

MARIE-LOUISE MICHEL*†, PATRIZIA PONTISSO‡, ELIANE SOBCZAK*, YVES MALPIÈCE*, ROLF E. STREECK*, AND PIERRE TIOLLAIS†

*Groupement de Génie Génétique and †Unité de Recombinaison et Expression Génétique (Institut National de la Santé et de la Recherche Medicale U.163, Centre National de la Recherche Scientifique LA 271), Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15 France; and ‡Istituto di Clinica Medica 2, Università di Padova, 35100 Padova, Italy

Communicated by Baruch S. Blumberg, August 16, 1984

ABSTRACT **A recombinant plasmid (pSVS dhfr) encoding the pre-S region and the *S* gene of human hepatitis B virus (HBV) and murine dihydrofolate reductase (DHFR) cDNA has been used for the transfection of Chinese hamster ovary (CHO) DHFR⁻ cells. Selection of clones resistant to methotrexate has permitted amplification of HBV sequences and an increase in production of hepatitis B surface antigen (HBsAg). HBV-specific transcripts have been characterized. The HBsAg 22-nm particles contain a receptor for polymerized human serum albumin (pHSA) and elicit in animals the synthesis of antireceptor antibodies. This property is ascribed to a 34,000-dalton polypeptide in the particles, which is most likely encoded by the *S* gene and part of the pre-S region. Especially because the pHSA receptor is most abundantly present on the virion and because, in hepatitis B infection, the appearance of anti-pHSA receptor antibodies seems to be a highly reliable criterion for viral clearance, the HBsAg particles obtained may constitute a particularly efficient vaccine.**

Sera of hepatitis B virus (HBV) chronic carriers contain empty viral envelopes (22-nm spherical particles and filaments) and occasionally complete infectious virions. The viral envelope carries the hepatitis B surface antigen (HBsAg), and the presence of virions is correlated with the detection of a soluble antigen, the e antigen (HBeAg).

The polypeptide composition of the viral envelope has been extensively studied. It consists of a major polypeptide present in two forms, glycosylated (GP27) and nonglycosylated (P24), and at least one minor polypeptide present in two forms (GP33 and GP36), differing in the extent of glycosylation (1). The amount of GP33 and GP36 in the envelope is variable: it may reach >10% of the envelope proteins in HBeAg positive antisera, while it comprises <1% in HBeAg negative antisera (1). The major polypeptide is encoded by the *S* gene, whereas the minor polypeptide is encoded by the *S* gene and a part of the pre-S region (2). This polypeptide carries a receptor for polymerized human serum albumin (pHSA) and could play a role in the penetration of the virus into the hepatocyte (3, 4).

The appearance of antibodies to pHSA receptor in the serum is observed during recovery from hepatitis B, characterized by the seroconversion HBeAg/anti-HBe antibodies, but never during the evolution toward chronicity (5, 6). This suggests that antibodies to pHSA receptor are important for viral clearance. A hepatitis B vaccine carrying the pHSA receptor therefore would probably be particularly efficacious.

We have constructed a recombinant plasmid containing the *S* gene and the pre-S region of HBV and the dihydrofolate reductase (DHFR) cDNA. After transfection of Chinese hamster ovary (CHO) DHFR⁻ cells with this plasmid, selection of methotrexate-resistant clones resulted in a large increase in the synthesis of HBsAg 22-nm particles carrying the pHSA receptor.

MATERIALS AND METHODS

Cell Culture and Transfection. CHO DHFR⁻ cells (kindly donated by L. A. Chasin) were propagated as described by Urlaub and Chasin (7). Growth in non-selective conditions was in Ham's F-12 medium supplemented with 4% each of newborn and fetal calf serum. CHO DHFR⁺ transformants were selected in Dulbecco's modified Eagle's minimal medium supplemented with 10% fetal calf serum and proline at 35 μg/ml.

Transfection of cells was performed according to the procedure of Graham and Van der Eb (8) as modified by Parker and Stark (9), except that carrier DNA was omitted. After growth for 3 days in non-selective medium, cells were subcultured (1:10) into selective medium. Colonies appearing 10–15 days later were isolated with cloning cylinders.

RNA Analysis. Approximately 10^8 cells were rinsed with phosphate-buffered saline (P_i/NaCl), scraped with a rubber policeman, and lysed with 3 M LiCl/6 M urea essentially as described by Auffray and Rougeon (10). Poly(A)⁺ RNA was purified from total RNA by oligo(dT) cellulose chromatography (11). For RNA blots, RNA was denatured with formaldehyde according to Lehrach *et al.* (12), electrophoresed through a 1.2% agarose gel, transferred to nitrocellulose and hybridized to the HBV 2.3-kilobase (kb) *Bgl* II-A fragment (specific activity, 10^8 cpm/μg) as described by Thomas (13). For S1 nuclease mapping analysis, the 0.8-kb *Xho* I/*Pvu* II fragment was used as radioactive probe. pSVS-dhfr was linearized with *Xho* I, dephosphorylated with bacterial alkaline phosphatase, and labeled with T4 polynucleotide kinase (Boehringer) and [γ-^{32}P]ATP (Amersham). After digestion with *Pvu* II, the shorter fragment was purified by acrylamide gel electrophoresis. The probe was hybridized with total cellular RNA and was digested with S1 nuclease as described by Berk and Sharp (14).

Purification of HBsAg. HBsAg was purified from cell culture supernatants harvested at 24-hr intervals from a high-producer clone. Pooled supernatants were clarified and 45% $(NH_4)_2SO_4$ (pH 7.5) was added. The precipitate was collected by centrifugation (15 min at 10,000 rpm) and the pellets were dissolved in a minimum volume of buffer A (10 mM Tris·HCl pH 7.5/150 mM NaCl/1 mM EDTA). After exhaustive dialysis against buffer A, CsCl was added to a density of

Abbreviations: DHFR, dihydrofolate reductase; HBV, human hepatitis B virus; HBsAg, hepatitis B surface antigen; pHSA, polymerized human serum albumin; pBSA, polymerized bovine serum albumin; HBeAg, hepatitis B e antigen; kb, kilobase(s); SV40, simian virus 40.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

1.20 g/cm^3, and two successive runs of isopycnic centrifugation were performed at 40,000 rpm for 24 hr in a 60 Ti rotor (Beckman) at 15°C. Fractions were collected from the top and were assayed for HBsAg. Peak fractions were pooled and dialyzed at 4°C against buffer A.

***In Vivo* Labeling and Immunoprecipitation of HBsAg.** CHO clones were maintained for 12 hr in methionine-depleted medium, followed by labeling for 6 hr with [^{35}S]methionine (150 μCi/ml) (Amersham; 1.2 mCi/mM; 1 Ci = 37 GBq). For glycosylation studies, cells were preincubated for 1 hr in the presence of tunicamycin (10 μg/ml) and radiolabeled in the presence of the same concentration of tunicamycin. Supernatants of transfected and nontransfected control cells were incubated overnight at 20°C with rabbit anti-HBs antiserum (final dilution, 1:40) with gentle agitation. They were then passed through a 0.5-ml column of protein-A Sepharose (Pharmacia) in 10 mM Tris·HCl, pH 7.4/150 mM NaCl/0.5% Nonidet P-40. After extensive washing of the column with the same buffer and finally with water, the immunoprecipitated proteins were eluted by boiling protein-A Sepharose with the sample buffer used for $NaDodSO_4$/PAGE (15). After electrophoresis, the gel was soaked in 1 M sodium salicylate (16), dried, and exposed to Kodak X-Omat R film at −70°C.

Immunization of Mice. Mice were immunized to determine the ED_{50} (median effective dose) in accord with OMS recommendations (17). Three groups of 20 female BALB/c mice were injected intraperitoneally on day 0 with 1 ml of Tris·HCl buffer containing 0.1% aluminium hydroxide and serial dilutions of HBsAg particles from either CHO cells or human serum (HEVAC-B vaccine, Institut Pasteur Production, France). As a control, five mice received 1 ml of diluant. Mice were bled on day 28 and antisera were individually checked for anti-HBs antibodies. A statistical analysis of the immune response was performed according to Finney (18).

pHSA Receptor Activity of HBsAg Particles. Two techniques were used. (*i*) Passive hemagglutination of sheep erythrocytes coated with pHSA was carried out as described (5). Briefly, sheep erythrocytes were coated with pHSA in 2.5% glutaraldehyde and adjusted to a 1% suspension in P_i/NaCl with 1% fetal calf serum and 1% sucrose. Twenty-five microliters of this suspension was incubated at room temperature for 1 hr in microplates with serial 2-fold dilutions of each HBsAg preparation, previously adjusted to the same protein concentration. Titers of pHSA binding activity were expressed as the highest dilution showing hemagglutination. (*ii*) Polyalbumin binding was also studied by solid phase RIA (19). Human serum albumin was polymerized by 2.5% glutaraldehyde treatment and the solution was then chromatographed on a Sephadex G-200 column in P_i/NaCl. The leading protein peak, containing the polymerized albumin, was pooled and used for coating polyvinyl microplates (50 μg/ml in carbonate buffer, pH 9.6). After 18 hr of incubation at room temperature, plates were washed with P_i/NaCl containing 0.05% Tween 20 and then were incubated for 18 hr with 10% fetal calf serum in carbonate buffer (pH 9.6). Control plates, coated with polymerized bovine serum albumin (pBSA), were also set up to assess the specificity of the receptor activity. Fifty microliters of the different HBsAg preparations were then added overnight at room temperature, and pHSA receptor associated with HBsAg was revealed by the addition of ^{125}I-labeled anti-HBs followed by another incubation for 4 hr at 37°C.

Hemagglutination Inhibition. To investigate the anti-receptor activity of antisera obtained in mice immunized with HBsAg, hemagglutination inhibition experiments were performed as follows: HBsAg of known hemagglutination titer (1:64) was mixed with an equal volume of each antiserum before testing in the hemagglutination assay. The results were expressed as percentage inhibition of pHSA receptor activity.

RESULTS

Transfection of CHO DHFR$^-$ Cells and Gene Amplification. The plasmid pSVS dhfr used for the transfection of CHO DHFR$^-$ cells carries two transcription units in a tandem arrangement (Fig. 1). The first unit consists of the HBV 2.3-kb *Bgl* II A DNA fragment—including the pre-S region, the *S* gene, and the HBsAg mRNA polyadenylylation site (22, 23)—placed downstream of the simian virus 40 (SV40) early promoter. The second unit consists of murine DHFR cDNA placed under the control of the MMTV-LTR promoter, the SV40 t-antigen splice site and the SV40 early mRNA polyadenylylation site (21).

CHO DHFR$^-$ cells were transfected by pSVS dhfr, DHFR$^+$ cells were selected, and HBsAg was assayed in the cell culture supernatant, using a radioimmunoassay (AUSRIA II, Abbott). Clones producing HBsAg were chosen for gene amplification and resistant methotrexate-cells were selected. A significant increase of HBsAg synthesis was observed (unpublished observation). One clone (37BA5R50) producing ≈1 μg per 10^6 cells per day was chosen for further study.

The organization of amplified sequences was studied by Southern blot analysis, using either HBV DNA or DHFR cDNA as probes. Complex patterns were observed in both cases, suggesting that DNA rearrangement occurred both in the initial transfection step as well as during amplification. Dot-blot hybridization of cytoplasmic RNA from methotrexate-resistant clones revealed a perfect proportionality between the level of HBsAg synthesis and the cellular concentration of HBV-specific RNA (unpublished observations).

Transcription of the HBV Sequences. The HBV-specific transcripts were studied by RNA blot analysis. Two HBV-specific poly(A)$^+$ RNAs were detected, a major one of 2.1 kb and a minor one of 2.5 kb (Fig. 2*a*). Initiation sites of transcription were determined by S1 nuclease mapping (Fig. 2*b*), using as a probe the 0.8-kb *Pvu* II/*Xho* I fragment comprising the SV40 promoter and most of the pre-S region. The 5′-end of the major RNA was localized in the sequence A-A-C-A (position 3154–3157) of the pre-S region (Fig. 2*c*). The second band detected in the S1 nuclease analysis is in agree-

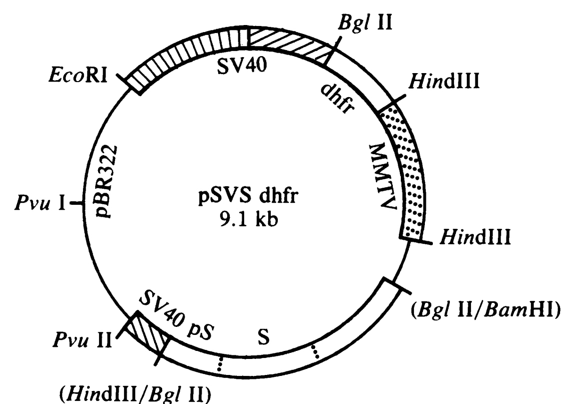

FIG. 1. Structure of pSVS-dhfr. pSVSdhfr was derived from pSVH4 (20) and pMTVdhfr (21) (a gift of G. Ringold). HBV and DHFR sequences are indicated by open segments, SV40 sequences by hatched segments, and pBR322 sequences by lines. The SV40 DNA preceding the HBV segments corresponds to the 360-base-pair *Pvu* II/*Hin*dIII fragment carrying the early promoter, which was fused to the 2.3-kb *Bgl* II-A fragment from HBV DNA by blunt-end ligation at the *Hin*dIII site. The MMTV and SV40 sequences flanking the DHFR cDNA are those present in pMTVdhfr. The pBR322 sequences correspond to the *Eco*RI/*Pvu* II fragment carrying the β-lactamase gene and the origin of replication, and to the 346-base-pair *Bam*HI/*Hin*dIII fragment.

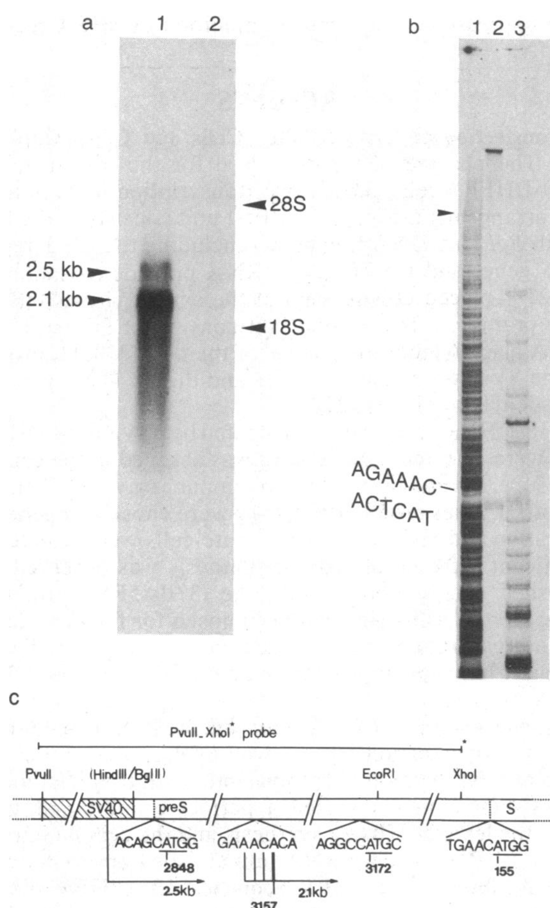

FIG. 2. Analysis of HBsAg specific mRNA. (*a*) RNA blot analysis: poly(A)$^+$ RNA from clone 37BA5R50 (lane 1) and CHO DHFR$^-$ cells (lane 2) was denatured, submitted to electrophoresis on a 1.2% agarose gel, and hybridized to the 2.3-kb *Bgl* II-A fragment. 18S and 28S rRNAs were used as size markers. (*b*) S1 nuclease mapping: the 0.8-kb *Pvu* II/*Xho* I fragment from pSVSdhfr was labeled at the *Xho* I site and was either submitted to sequencing reactions or hybridized to cytoplasmic RNA from clone 37BA5R50 and submitted to S1 nuclease digestion. Lane 1, piperidine formate reaction (24); lane 2, S1 nulcease digestion; lane 3, $KMnO_4$ reaction (25). The DNA sequence given is that of nucleotides 3151–3162 of the pre-S region, which is in agreement with the sequencing reactions. Arrow indicates the position of the minor initiation site of transcription. (*c*) Schematic representation of the SV40–HBV junction and the pre-S region. Nucleotide sequences around the three in-phase ATG codons (underlined) of the pre-S region and the *S* gene are given. Possible initiation sites of transcription are indicated. Numbering of the HBV sequence corresponds to that of Tiollais *et al* (22).

ment with an initiation of the 2.5-kb RNA in the SV40 sequence, but its position has not been mapped precisely.

Structure and Immunogenicity of the HBsAg 22-nm Particles. HBsAg purified from the cell culture supernatant of clone 37BA5R50 consisted of homogeneous 22-nm particles with a density of 1.21 g/cm^3 in CsCl; no filaments could be detected (Fig. 3). NaDodSO$_4$/PAGE of purified particles revealed three bands corresponding to polypeptides with apparent molecular sizes of 22, 26, and 34 kDa (Fig. 4*a*). According to results shown by silver staining (26), these polypeptides were present in relative amounts of $\approx$45%, $\approx$18%, and $\approx$35%, respectively. Immunoprecipitation of ^{35}S-labeled particles with rabbit anti-HBs antiserum, followed by PAGE and autoradiography, revealed two bands at 22 and 26 kDa and a large heterogenous band at a mean position of 34 kDa (Fig. 4*b*). All these polypeptides were therefore constituents of the HBsAg particles.

To study the glycosylation, cells were incubated in the presence of tunicamycin (27). The 26-kDa band disappeared, whereas the intensity of the 22-kDa band increased. The broad band at 34 kDa gave rise to two bands, one of them at a lower position (Fig. 4b). This showed that the polypeptides of 26 and 34 kDa are both glycosylated. The heterogeneity of the band at 34 kDa could be due to a difference in the extent of glycosylation.

The immunogenicity of the HBsAg particles synthesized in CHO cells was studied in BALB/c mice and compared with HBsAg particles purified from human serum (HEVAC-B, vaccine). As shown in Table 1, the efficiencies of the two HBsAg preparations were identical: anti-HBs seroconversion was noted in 50% of the mice (ED$_{50}$) after immunization with 0.04 and 0.03 μg of CHO particles and human particles, respectively. The relative potency of the CHO particles and the HEVAC-B vaccine is 0.7 (0.4 to 1.2) with an interval of confidence of 95%.

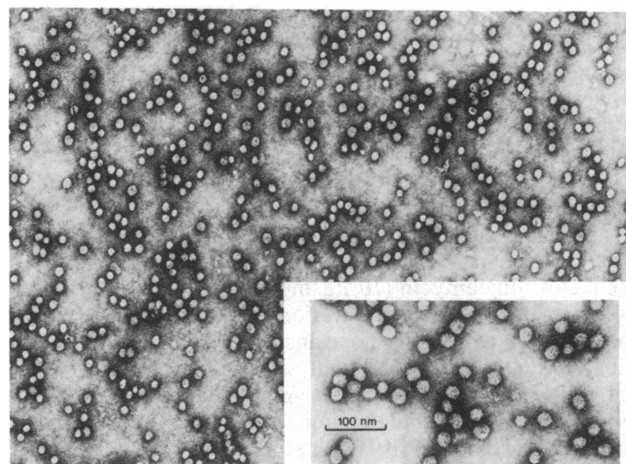

FIG. 3. HBsAg particles synthesized in CHO cells. Purified HBsAg particles from clone 37BA5R50 were adsorbed on collodium-coated grids, stained with uranyl acetate, and visualized by electron microscopy. (×22,000; *Inset*, ×33,000.)

pHSA Receptor Activity of the HBsAg 22-nm Particles. Since the pSVS dhfr plasmid contained both the pre-S region and the *S* gene, we tested the capacity of CHO cells transfected with pSVS-dhfr to synthesize HBsAg particles carry-

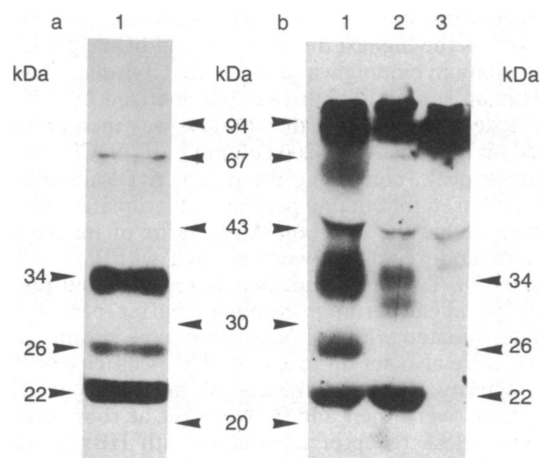

FIG. 4. Electrophoretic analysis of the HBsAg particle polypeptides. (*a*) Polypeptides of purified particles from clone 37BA5R50 visualized by silver staining. (*b*) Autoradiography of ^{35}S-labeled polypeptide material from CHO supernatants immunoprecipitated with a rabbit anti-HBs antiserum. Lane 1, clone 37BA5R50; lane 2, clone 37BA5R50 in the presence of tunicamycin; lane 3, CHO DHFR$^-$ cell line.

Table 1. Anti-HBs immune response

HBsAg injected, μg	CHO HBsAg particles: Number of seroconverted mice (titer > 50 RIA units)	CHO HBsAg particles: anti-HBs titer GMT	Hevac-B vaccine: Number of seroconverted mice (titer > 50 RIA units)	Hevac-B vaccine: anti-HBs titer GMT
0.312	20/20	505	20/20	388
0.078	14/20	87	19/20	212
0.019	6/20	24	6/20	24
0	—	—	—	—
	ED_{50}, 0.04 μg		ED_{50}, 0.03 μg	

GMT, geometric mean titer in RIA units (AUSAB, Abbott); 3.5 RIA units are equivalent to 1 milliinternational unit. ED_{50}, effective dose required to seroconvert 50% of mice.

ing the pHSA receptor. Two different methods—hemagglutination of sheep erythrocytes coated with pHSA and solid-phase RIA—were used. A pHSA binding activity specific for human albumin was detected (Table 2). pBSA did not bind at all. Moreover, purified HBsAg particles were able to elicit antibodies to the pHSA receptor in mice (Table 3). This was demonstrated by the complete inhibition of the hemagglutination assay by the antisera of mice immunized with the CHO HBsAg particles. Under the same conditions, anti-pHSA receptor antibodies were not detected in mice immunized with human particles (HEVAC-B).

To determine the specificity of the anti-pHSA receptor antibodies, undiluted antiserum of mice immunized with CHO HBsAg particles was absorbed by HBsAg particles of HBeAg positive human antisera (pHSA receptor positive by RIA). The anti-pHSA receptor activity, tested by inhibition of hemagglutination, shifted from 100% to 0% after four steps of absorption. In parallel mice antisera incubated with HBsAg particles of HBeAg positive human antisera treated with Pronase (and thus devoid of pHSA receptor activity) conserved an anti-pHSA receptor activity of 100%.

DISCUSSION

Transfection of CHO $DHFR^-$ cells with HBV–DHFR recombinant plasmid and selection of methotrexate-resistant clones have permitted amplification of the HBV sequences with a significant attendant increase in HBsAg synthesis.

The HBsAg particles produced from the CHO clones appeared to be rather homogeneous and had the same diameter and the same density in CsCl as HBsAg 22-nm particles from human serum. They contained three polypeptides. Two of them, with apparent molecular sizes of 22 and 26 kDa, can be identified as the two forms of the major envelope polypeptide, which is encoded by the *S* gene (22). The analysis of immunoprecipitated CHO particles has shown that the 34-kDa polypeptide is also a constituent of these particles. This suggests that this polypeptide corresponds to the HBV GP33 or GP36 and is encoded by the *S* gene and a part of the pre-S region. The determination of the NH_2 terminus sequence of the 34-kDa polypeptide will be necessary to confirm initiation at the second AUG codon of the pre-S region (position 3172, Fig. 2*c*). Moreover, the radiolabeling of cellular proteins with or without tunicamycin clearly showed that polypeptide glycosylation is not required for the secretion of HBsAg particles. The absence of HBsAg secretion in yeast (28, 29) is therefore not explained by a glycosylation defect.

Two HBV-specific transcripts were characterized. The start initiation site of the major 2.1-kb RNA was mapped in the pre-S region around nucleotides 3154–3157. The CAC sequence at this position matches perfectly with the consensus sequence pyApy of capping sites (30). This mapping is in agreement with the results of Cattaneo *et al.* (31) and Standring *et al.* (32). The 2.1-kb RNA can code for both the major polypeptide as well as for the 34-kDa polypeptide. The presumptive start codon of this polypeptide would be the first ATG to occur, about 15 nucleotides from its 5′ end. Examples are known of viral mRNAs carrying one initiator codon close to the 5′ end and a second one in phase further downstream (33). The 2.5-kb minor mRNA is probably initiated in the SV40 DNA sequences. Its coding capacity is unknown. It could theoretically code for three polypeptides initiated at positions 2848, 3172, and 155, respectively (Fig. 2*c*). No polypeptide corresponding to an initiation of translation at position 2848 was detected. This is unexpected because the A-G-C-A-T-G-G sequence at this position is a strong initiation site of translation (33). The relatively large amount of 34-kDa polypeptide (up to 50% of the envelope proteins) in CHO cells is difficult to explain. We have not determined whether this polypeptide is translated from the 2.1-kb or the 2.5-kb RNA. A favorable secondary structure of the 5′ end of the 2.5-kb RNA could enhance initiation at the AUG codon of the 34-kDa polypeptide. The large amount of this polypeptide could also be due to an excess of 2.1-kb RNA joined with a coupling between the level of transcription and

Table 2. pHSA binding activity on HBsAg particles

	Cell culture supernatant: Clone 37BA5R50 RIA	Clone 37BA5R50 HA	CHO $DHFR^-$ cell line RIA	CHO $DHFR^-$ cell line HA	Human serum: HBsAg positive, HBeAg positive RIA	HBsAg positive, HBeAg positive HA	Normal RIA	Normal HA
pHSA	9742	128	72	Neg	10,374	256	200	Neg
pBSA	112	—	54	—	158	—	36	—

Results are expressed in cpm for solid phase RIA and in hemagglutination titer (HA) as described in *Materials and Methods*. Neg, negative.

Table 3. Anti-pHSA receptor immune response

HBsAg injected, μg	CHO HBsAg particles: anti-pHSA receptor, antibody titer (HA)	CHO HBsAg particles: anti-pHSA receptor activity, %
0.312	16	100
0.078	4	100
0.019	2	100
0	0	0

Anti-pHSA receptor activities were determined on pooled sera. Results of anti-pHSA receptor activity were expressed as the percent inhibition of hemagglutination (HA). Purified HBsAg having a pHSA receptor hemagglutination titer of 1:64 was mixed with an equal volume of undiluted inhibitor. Results are expressed as pHSA receptor titer of HBsAg with inhibitor/pHSA receptor titer of HBsAg with buffer. As a control, identical quantities of Hevac-B vaccine were used. In each case, anti-pHSA receptor antibody titer and activity were zero.

the efficacy of translation at this initiation codon. The high level of mRNA synthesis in CHO cells could be related to the use of the SV40 enhancer sequences and the gene amplification process. We can note that a construction containing a comparable fragment of HBV placed downstream of promoter–enhancer sequences of murine sarcoma virus (34) or bovine papilloma virus (35) also directed the synthesis of a 34-kb polypeptide. The relative excess of GP33 and GP36 in particles of HBeAg positive patients could also be explained by an increase of HBV mRNAs during attendant virus replication.

The HBsAg particles synthesized in CHO cells carry a receptor for pHSA. This receptor is species specific, as indicated by its inability to bind to the pBSA. Contrary to the HEVAC-B vaccine, the CHO particles elicited anti-pHSA receptor antibodies in mice. Absorption experiments showed that these antibodies specifically reacted with HBsAg particles of human origin. This discrepancy is ascribed to the absence of the 34-kDa polypeptide in HEVAC-B particles purified from HBeAg negative carriers (data not shown). Machida *et al.* (3, 4) have shown that the receptor activity is carried by the minor polypeptide of the envelope. This strongly suggests that the receptor activity present on the HBsAg particles produced by the CHO cells was present on the 34-kDa polypeptide. The structure of the particles of CHO origin is therefore comparable to that of particles present in the serum during viral multiplication; i.e., presence of both HBsAg and receptor activities.

Synthesis of HBsAg 22-nm particles in animal cells (36) or yeast (28, 29) constitutes, for the near future, the most appropriate alternative to the present hepatitis B vaccine. The final choice of the source of HBsAg will depend mainly on three parameters: the immunogenicity of the particles, the possibilities for industrial transfer of the procedure of HBsAg production, and the safety of the final product.

The immunogenic value of the particles of CHO origin was studied in mice. The dose necessary to seroconvert 50% of mice to anti-HBs was identical to that of particles of human origin. The good correlation between the immunogenicity of HBsAg in animals and humans [§] suggests therefore that the particles from CHO cells may elicit a strong immune response in man. The capacities of these particles to induce antibodies to the pHSA receptor could be an additional argument for their use as a vaccine.

[§]Goudeau, A., Dubois, F. & Dubois, M. C. (1984) The International Symposium on Viral Hepatitis, San Francisco.

We thank G. Ringold for pMTV dhfr plasmid, L. Chasin for CHO DHFR$^-$ cells, C. Mazert for mice immunization, F. Chisari for reviewing the text, and L. M. Da for typing the manuscript. This work was supported by the Institut Mérieux and the Institut Pasteur Production.

1. Stibbe, W. & Gerlich, W. H. (1982) *Virology* **123**, 436–442.
2. Stibbe, W. & Gerlich, W. H. (1983) *J. Virol.* **46**, 626–628.
3. Machida, A., Kishimoto, S., Ohnuma, H., Miyamoto, H., Baba, K., Oda, K., Nakamura, T., Miyakawa, Y. & Mayumi, M. (1983) *Gastroenterology* **85**, 268–274.
4. Machida, A., Kishimoto, H., Ohnuma, H., Baba, K., Ito, Y., Miyamoto, H., Funatsu, G., Oda, K., Usuda, S., Togami, S., Nakamura, T., Miyakawa, Y. & Mayumi, M. (1984) *Gastroenterology* **86**, 910–918.
5. Pontisso, P., Alberti, A., Bortolotti, F. & Realdi, G. (1983) *Gastroenterology* **84**, 220–226.
6. Alberti, A., Pontisso, P., Schiavon, E. & Realdi, G. (1984) *Hepatology* **4**, 220–226.
7. Urlaub, G. & Chasin, L. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4216–4220.
8. Graham, R. & Van der Eb, A. (1973) *Virology* **52**, 456–467.
9. Parker, B. A. & Stark, G. R. (1979) *J. Virol.* **31**, 360–369.
10. Auffray, C. & Rougeon, F. (1980) *Eur. J. Biochem.* **107**, 303–314.
11. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
12. Lerhach, H., Diamond, D., Wozney, J. M. & Boedtker, R. (1977) *Biochemistry* **16**, 4743–4752.
13. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
14. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12**, 721–732.
15. Laemmli, U. K. (1977) *Nature (London)* **227**, 680–685.
16. Chamberlain, G. T. (1979) *Anal. Biochem.* **98**, 132–135.
17. World Health Organization Technical Report Series (1981) no. 658.
18. Finney, D. J. (1971) *Statistical Method in Biological Assay* (Griffin, London), 2nd Ed.
19. Pontisso, P., Alberti, A., Schiavon, E., Tremolada, F., Bortolotti, F. & Realdi, G. (1983) *J. Virol. Meth.* **6**, 151–159.
20. Malpiece, Y., Michel, M. L., Carloni, G., Revel, M., Tiollais, P. & Weissenbach, J. (1983) *Nucleic Acids Res.* **11**, 4645–4654.
21. Lee, F., Mulligan, R., Berg, P. & Ringold, G. (1981) *Nature (London)* **294**, 228–232.
22. Tiollais, P., Charnay, P. & Vyas, G. N. (1981) *Science* **213**, 406–411.
23. Pourcel, C., Louise, A., Gervais, M., Chenciner, N., Dubois, M. F. & Tiollais, P. (1982) *J. Virol.* **42**, 100–105.
24. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–567.
25. Rubin, C. M. & Schmid, C. W. (1980) *Nucleic Acids Res.* **8**, 4613–4619.
26. Oakley, B. R., Kirsch, D. R. & Morris, N. R. (1980) *Anal. Biochem.* **105**, 361–363.
27. Takatsuki, A. & Tamura, G. (1971) *J. Antibiot.* **24**, 785–794.
28. Valenzuela, P., Medina, A. & Rutter, W. J. (1982) *Nature (London)* **298**, 347–350.
29. Miyanohara, A., Toh-e, A., Nozaki, C., Hamada, F., Ohtomo, N. & Matsubara, K. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1–5.
30. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50**, 349–383.
31. Cattaneo, R., Will, H., Hernandez, N. & Schaller, H. (1983) *Nature (London)* **305**, 336–338.
32. Standring, D. N., Rutter, W. J., Varmus, H. E. & Don Ganem (1984) *J. Virol.* **50**, 563–571.
33. Kozak, M. (1983) *Microbiol. Rev.* **47**, 1–45.
34. Stratowa, C., Doehmer, J., Wang, Y. & Hofschneider, P. H. (1982) *EMBO J.* **1**, 1573–1578.
35. Wang, Y., Stratowa, C., Schaefer-Ridder, M., Doehmer, J. & Hofschneider, P. H. (1983) *Mol. Cell. Biol.* **3**, 1032–1039.
36. Dubois, M. F., Pourcel, C., Rousset, S., Chany, C. & Tiollais, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4549–4553.