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HEPATITIS B VIRUS TRANSCRIPTS IN A HUMAN HEPATOMA CELL LINE, HEP 3B

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SUMMARY Hep 3B, a human hepatoma cell line was examined for its RNA hybridizable to the hepatitis B virus sequence. Using probes that covered different regions of the hepatitis B virus genome, five species of RNA were observed of sizes 4.0, 3.3, 2.9, 2.6 and 2.2 kilobases. The RNAs covered surface antigen gene, pre-S and X regions. None of them had a core antigen sequence. RNA with a 4.0 kilobase size was the most abundant. Using S1 nuclease analysis, its 5′ end of hepatitis B virus sequence was mapped at pre-S region and its 3′ end of viral sequence was mapped at DR region.

Hepatitis B virus (HBV) is considered to have an etiological relationship to the development of primary liver carcinoma (1,2). Many attempts have been made to search for HBV genes and their products as related to transformation (3). However, the relationship of HBV and the transformation of hepatocytes is still unknown at the present time. Hep 3B is a well-differentiated human hepatoma cell line with HBV DNA integrated (4). This cell line can serve as an ideal experimental system for the study of control of HBV transcription in the absence of viral replication. The cell lines can also provide a system for search for the possible HBV gene products related to transformation. In this study, the HBV RNA produced in Hep 3B cell line was examined and the region of HBV genome being transcribed was defined.

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MATERIALS AND METHODS

Cell line --- Hep 3B cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum. For RNA preparation, the culture was shifted to DMEM without serum supplement to enrich it for HBs Ag expression (C.K. Chou et al., manuscript in preparation).

Isolation of cellular DNA and RNA --- High molecular weight DNA was prepared by the method of Blin and Stafford (5). The total RNA was extracted by the guanidinium/cesium chloride method (6). Poly (A^+) RNA was purified by two sequential frationations using oligo(dT)-cellulose chromatography as described by Aviv and Leder (7).

Electrophoresis and detection of HBV DNA and RNA --- DNA electrophoresis, and the transfer of DNA to nitrocellulose paper, then hybridization were performed as described by Southern (8). RNA was denatured by glyoxal, electrophoresed, transferred to nitrocellulose paper and detected with [^{32}P] labeled nick-translated HBV probe as described (9,10).

S1 nuclease analysis --- Total RNA (60ug) was hybridized with 10ng of HBV DNA fragment (as shown in Fig. 4I) and digested with S1 nuclease as described (11,12). The nucleic acid was precipitated and resuspended in 20 ul of 0.1 N NaOH, 10 mM EDTA, heated at 68°C for 10 min, precipitated with ethanol and denatured by addition of glyoxal (13). The protected DNA was fractionated by electrophoresis on a 1.2% agarose gel, transferred to nitrocellulose paper and hybridized with a [^{32}P] labeled nick-translated DNA probe prepared from pTWL1 HBV insert (14).

RESULTS AND DISCUSSION

Southern blot analysis of HBV DNA --- Fig. 1 shows the restrictive fragments of HindIII, EcoR1 and BamH1 digested HBV DNA in Hep 3B cell line. In BamH1 digested DNA, there were several HBV hybridizable fragments, with one predominating which was about the size of 1.4 kb; this fragment hybridized strongly with DNA probe containing surface antigen region (data not shown). Among HBV strains, only DNA of adw subtype can be cleaved with BamH1 restriction enzyme to produce a fragment 1.4 kb in size containing the surface antigen gene (14). In this study, Hep 3B cell line does show a BamH1 1.4 kb fragment that is hybridizable with surface antigen probe. This strongly suggests that the HBV genome in Hep 3B is derived from adw subtype.

Northern blot analysis of HBV RNA --- Knowles et al. reported that Hep 3B cell line produces two major polypeptides of the hepatitis B virus surface antigen (15). To examine the nature of their RNA, the RNAs were hybridized to the entire HBV genome.

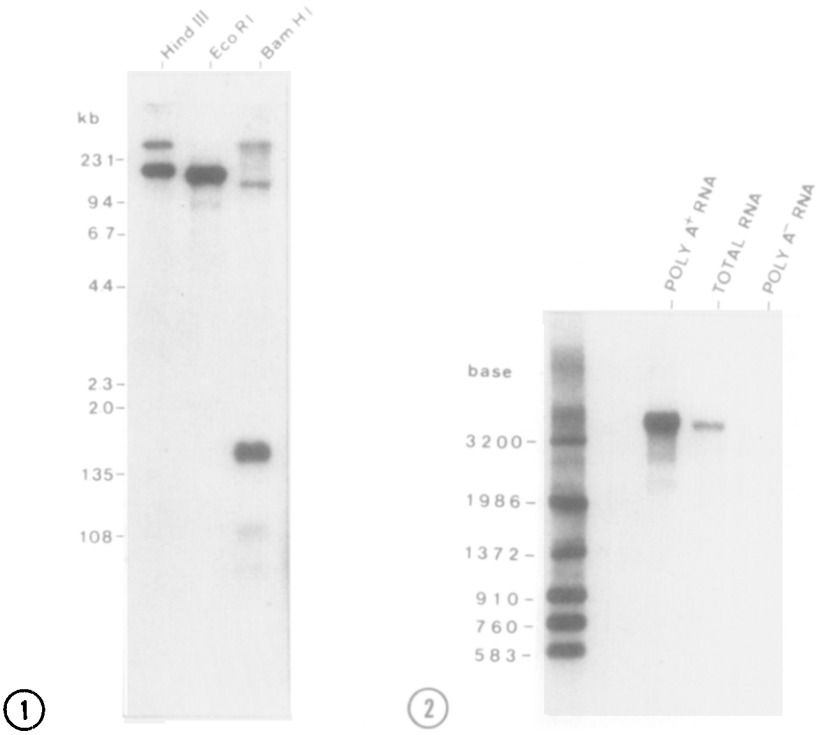

Fig. 1. Restriction pattern of DNA from Hep 3B detected with entire HBV genome as probe. Genomic DNA (10 ug) was digested with restriction endonucleases HindIII, EcoRl and BamHl and applied to a 0.8% agarose gel for electrophoresis. The DNA was denatured and transferred to nitrocellulose paper, then hybridized with nick-translated [^{32}P]DNA prepared from the entire HBV genome of pTWL1 (14).

Fig. 2. Northern blot analysis of RNA of Hep 3B with entire HBV genome as probe. 5 ug of poly (A^+) RNA, 20 ug of total RNA or 20 ug of poly (A^-) RNA were denatured with glyoxal and applied to a 1.2% agarose gel for electrophoresis. The RNA was transferred to nitrocellulose paper and hybridized with nick-translated [^{32}P] DNA prepared from the entire HBV genome of pTWL1.

Fig. 2 shows a major species of RNA, 4.0 kb in size, and 2 minor ones, 2.9 kb and 2.2 kb in size, were detected. To further characterize them, RNAs were hybridized with probes to surface antigen, core antigen, X region or pre-S1 region (Fig. 3I). Fig. 3II shows all three species of RNA -- 4.0 kb, 2.9 kb and 2.2 kb in size -- were hybridized with S or X probes (Fig. 3II, lane A and C). In contrast, when probing with HBV fragment that covers core antigen gene, none of the RNA was hybridized (Fig. 3II, lane E). Lane G shows when one used probe of pre-S1 region, the

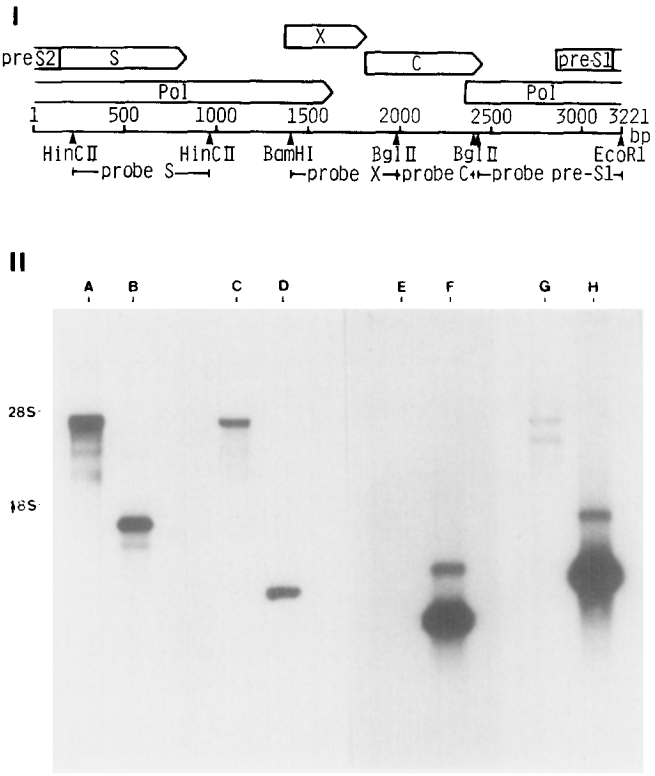

Fig. 3. (I) A linear form of HBV genome. The entire HBV genome was cut out from plasmid pTWL1 by restriction enzyme EcoRl and presented in a linear form. Open arrows represent known and predicated genes. The HincII fragment (nucleotide 219-963) served as S probe, the BamHl-BglII fragment (nucleotide 1402-1986) served as X probe, the BglII fragment (nucleotide 1986-2407) served as C probe and the BglII-EcoRl fragment (nucleotide 2433-3200) served as pre-S1 probe. The above fragments were subcloned into plasmid pSP65 and reisolated before used as a probe in part II. (II) Northern blot analysis of RNA of Hep 3B with different regions of HBV genome as probes. Blot hybridization was carried out as described in Fig. 2. The [^{32}P]DNA probes were prepared by nick translating the DNA inserts of various plasmids and are indicated as follows: lanes A and B hybridized with S probe, lanes C and D hybridized with X probe, lanes E and F with C probe, and lanes G and H with pre-S1 probe. Lanes A,C,E,G contained 3 ug of poly (A^+) RNA and lanes B,D,F,H served as control by hybridizing the probe to its own DNA (0.1 ug to 0.05 ug).

hybridization signal of 4.0 kb RNA was dramatically reduced. The result suggests that only a little of the pre-S1 region is present in this species of RNA. Two other species of RNA that also hybridized with this probe was in the sizes 3.3 kb and 2.6 kb. The fact that neither the 3.3 kb and 2.6 kb RNA were detected by the previous probes might be due to the abundance of the two

RNAs. The conclusion is that in the Hep 3B cell line, there are five detectable HBV RNA in the sizes of 4.0, 3.3, 2.9, 2.6, 2.2 kb. The major 4.0 kb HBV transcript covers the surface antigen gene, the X region and part of the pre-S region. The minor species of RNA of 2.9 kb and 2.2 kb in size cover surface antigen gene and X region and two other minor species, 3.3 kb and 2.6 kb, may cover most of the pre-S region. None of these transcripts possess core antigen sequences although from Southern blot analysis, DNA fragments hybridizable with core-specific DNA probe can be detected (data not shown).

S1 nuclease mapping analysis --- To get a more precise analysis of the HBV region in the major 4.0 kb transcript, S1 nuclease mapping was employed. Since the 4.0 kb RNA was at least 20-fold more abundant than other species, the minor species did not interfere with this analysis. From Southern blot analysis of HBV genome in Hep 3B, it was concluded that HBV genome in this cell line is derived from adw subtype. In order to maximize the homology between Hep 3B HBV transcripts and the cloned HBV genome employed in the S1 nuclease protection study, the cloned HBV DNA in this study was also derived from the adw subtype. The modified S1 nuclease analysis was followed by using [^{32}P]-labeled nick-translated HBV probe to study the protected DNA fragment to assure that every protected fragment had been detected. Fig. 4I illustrates the partial restriction map of HBV genome and the HBV DNA fragments used in this study. Fig. 4II shows that, when the EcoRl-EcoRl fragment containing the entire HBV genome was used to protect the Hep 3B RNA from S1 nuclease digestion, a fragment of 1790 bp was protected, indicating that only a portion of HBV genome was in 4.0 kb size transcript. To map this region, HBV fragments covering different portions of HBV genome were employed. When BamHl-BglII fragment (from nucleotide 1402 to

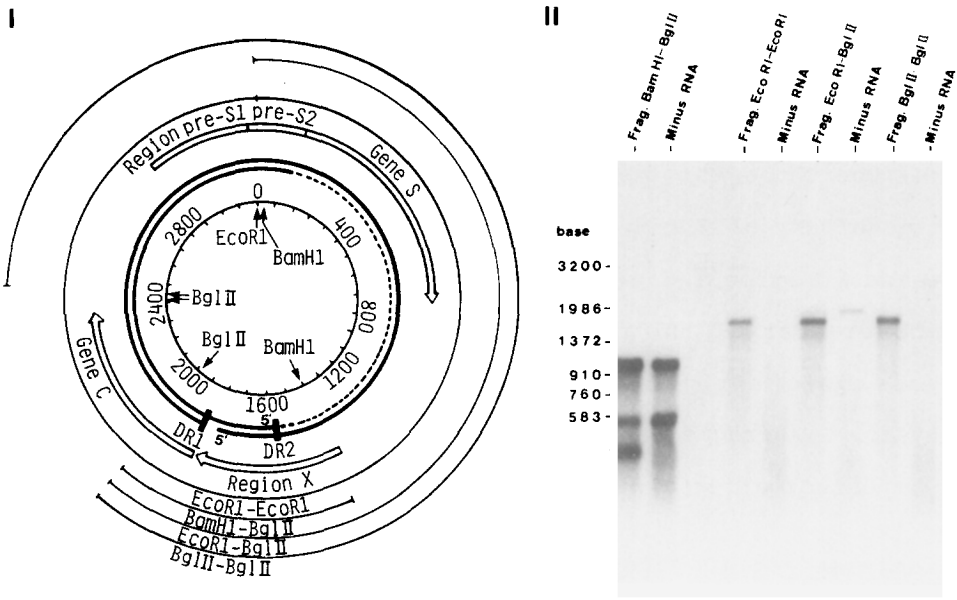

Fig. 4(I). Structure and partial restriction map of HBV genome of adw subtype. The coding regions for the hepatitis B surface antigen, core antigen, Pre-S1, pre-S2 and X regions are indicated. The thin lines marked EcoRl-EcoRl, BamHI-BglII, EcoRl-BglII and BglII-BglII are the DNA fragments used for S1 nuclease analysis. (II) S1 nuclease analysis of total cellular RNA from Hep 3B. The 60 ug of total cellular RNA was analyzed as described under "Materials and Methods". The minus RNA lane is the S1 nuclease analysis carried out under the same experimental conditions, but with no RNA present.

nucleotide 1986) was used, besides two fragments that were also present in the control (i.e. the lane of minus RNA) a third fragment of 390 bp in size was protected. The extra band appearing in this control lane might result from the incomplete denaturation of double stranded DNA. To orient the positon of 390 bp fragment, the fragment EcoRl-BglII--that is from nucleotide 1 to nucleotide 1986--was used in the analysis. A fragment of about 1790 nucleotides, the same size as that protected by the EcoRl-EcoRl fragment, was protected. The data indicates within the 4.0 kb size RNA, the 3′ end of HBV sequence mapped at 390 bp downstream of the 1400 nucleotide BamHl site, i.e. 1790 nucleotide on HBV map in Fig. 4I. To define the 5′ region of HBV sequence, a BglII-BglII fragment from nucleotide 2423 clockwise

to nucleotide 1986 was employed. The size of the protected fragment was very close to the fragment protected by the EcoRl-BglII fragment. This implies that the 5′ end of the HBV sequence is near the EcoRl site on the HBV map. So the 4.0 kb transcript contains the HBV sequence from approximately the EcoRI site to the region around nucleotide 1790 on the map. This is good agreement with the results obtained in Fig. 3.

The results of Sl nuclease analysis suggests that the 5′end of HBV sequence of the major 4.0 kb RNA is mapped to the region close to the EcoRl site at nucleotide 3200, a region homologous to the Simian virus 40 (SV40) late promoter (16). The 3′end of the HBV sequence on the 4.0 kb transcript is at about 1690 nucleotide on the HBV map, the region of DRl. Our analyses of the 4.0 kb transcript suggest it may be a hybrid of HBV and host RNA. It can be initiated at SV40-like late promoter in the pre-S region and transcribed through DR sequence and terminated at host genome. Alternatively, the integrated HBV sequences have been rearranged so that tandem inverted repeated configuration occurred. The 4.0 kb transcript can be the sum of the transcripts from tandem repeated viral sequences. We are currently investingating these possibilities. The large size and possibly fused transcript and various species of HBV RNA make the Hep 3B cell line an attractive system to study the transcriptional and translational control of HBV gene expression and to study the possible relationship between host and virus in tumorgenesis.

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REFERENCES

1. Szmuness, W. (1978) Prog. Med. Virol. 24, 40-65.
2. Beasley, R.P., Hwang, L.Y., Lin, C.C., and Chien, C.S. (1981) Lancet 2, 1129-1133.
3. Moriarty, A.M., Alexander, H., and Lerner, R.A. (1985) Science 227, 429-433.
4. Twist, E.M., Clark, H.F., Aden, D.P., Knowles, B.B., and Plotkin, S.A. (1981) J. Virol. 37, 239-243.
5. Blin, N., and Stafford, D.W. (1976) Nucleic Acids Res. 3, 2303-2308.
6. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular cloning, a laboratory manual, Cold Spring Harbor Laboratory.
7. Aviv, H., and Leder, P. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 1408-1412.
8. Southern, E. (1975) J. Mol. Biol. 98, 503-517.
9. Wahl, G.M., Padgett, R.A., and Stark, G.R. (1979) J. Biol. Chem. 254, 8679-8689.
10. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5201-5205.
11. Berk, A.J., and Sharp, P.A. (1977) Cell 12, 721-732.
12. Weaver, R.F., and Weissmann, C. (1979) Nucleic Acids Res. 7, 1175-1193.
13. McMaster, G.K., and Carmichael, G.G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4835-4838.
14. Lo, S.J., Lee, Y.-H. W., Chiou, J.-S., Ting, L.-P., Liu, W.-T., and Choo, K.-B., (1985) Biochem. Biophy. Res. Com. 129, 797-803.
15. Knowles, B.B., Howe, C.C., and Aden, D.P. (1980). Science 209, 497-499.
16. Cattaneo, R., Will, H., Hernandez, N., and Schaller, H. (1983) Nature 305, 336-338.