Core Particles of Hepatitis B Virus and Ground Squirrel Hepatitis Virus

II. Characterization of the Protein Kinase Reaction Associated with Ground Squirrel Hepatitis Virus and Hepatitis B Virus

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The recently described protein kinase activity in hepatitis B virus core antigen particles (Albin and Robinson, *J. Virol.* **34**:297-302, 1980) has been demonstrated here in the liver-derived core particles of ground squirrel hepatitis virus. Both protein kinase activities were initially associated with DNA polymerase-positive heavy core particles in CsCl density equilibrium gradients and shifted to polymerase-negative cores during the course of purification. The major core-associated polypeptide of each virus was the dominant species labeled. A variable number of other polypeptide species were also labeled by this reaction. Tryptic peptide mapping of both major and minor phosphorylated polypeptides of each virus resulted in similar patterns, suggesting that many of the sites of phosphorylation were the same in the components of each core particle. Hydrolysis of these phosphorylated core particles revealed a major phosphoamino acid as serine and a minor phosphoamino acid as threonine. The products of the protein kinase reaction in both human hepatitis B and ground squirrel hepatitis virus core particles, then, share many characteristics. The possible function(s) of this protein kinase activity is discussed in the light of similarly characterized activities in other animal viruses.

Hepatitis B virus (HBV) and the recently discovered ground squirrel hepatitis virus (GSHV) have been shown to share a number of important characteristics, which has resulted in their grouping as hepadna viruses (22a). The virions of each are spherical and possess an envelope of cross-reacting surface antigen determinants (6, 7, 9, 15). Peptide mapping of the major nonglycosylated surface antigen polypeptides of each has shown at least 25% structural homology (7). The region of the HBV genome encoding the major surface antigen polypeptide also hybridizes to a fragment of GSHV DNA (27). Both viruses possess a cross-reacting internal core antigen which is immunologically and structurally distinct from surface antigen (2, 8, 15). The major core antigen polypeptides of GSHV and HBV share more than half of their tryptic peptides (8). The core particles, or nucleocapsids, also contain e antigen (16, 17, 29), viral DNA (16, 21), and an endogenous DNA polymerase activity (11, 15, 22). The DNA of these viruses is circular and approximately 3,200 base pairs in length and can be linearized upon

heating (14, 21, 24, 27). The genomes are also partially double stranded and can be repaired by the endogenous DNA polymerase (14, 15, 21). Infection of ground squirrels and humans with their respective viruses often results in persistent surface antigen expression in the form of 22-nm spherical particles found in high concentrations in the blood. Virions are also found, but in much lower concentrations. The surface antigen particles in these persistent infections bear the same antigenic determinants as those of the virion envelope, but are often found in the absence of any other viral markers.

A protein kinase has been shown to be present in the 42-nm hepatitis B virions (or Dane particles) isolated from serum and the 28-nm HBV core particles obtained from Dane particles or liver, but not from 22-nm-diameter surface antigen particles (1). The major polypeptide of the virion core particle is the component most obviously phosphorylated, but several high-molecular-weight phosphoproteins have also been observed. Furthermore, the 20,600-dalton major core polypeptide is apparently cleaved, in the presence of antibody to hepatitis B core antigen, to polypeptides of 14,700 and 6,000 daltons. In this study it is shown that a protein kinase

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reaction is found in liver-derived GSHV core particles and that the tryptic peptides and amino acids phosphorylated in the core particles share many of the same characteristics as those similarly identified in HBV cores.

HBcAg and GSHcAg were isolated from livers selected as described (8). Core particles from freshly homogenized tissue were purified by two steps of CsCl density equilibrium centrifugation and an intervening step of rate-zonal sedimentation in CsCl as described (8). DNA polymerase and protein kinase assays and enzyme-linked immunosorbent assay, in addition to other measured parameters of each gradient fraction, were carried out as outlined in the legend to Fig. 1. The peak fraction of protein kinase activity, determined by assay of each gradient fraction, was used for preparing phosphorylated core polypeptides for further analysis (1). A portion of these peak fractions was diluted in phosphate-buffered saline (pH 7.5) and pelleted overnight, at 50,000 rpm, at 10°C in an SW60 rotor. The supernatants were gently decanted, and the protein kinase reaction was carried out (1) in 75 μ l using 200 μ Ci of [γ -³²P]ATP (Amersham). After 1 h in a 37°C water bath, the reaction was terminated by addition of 5 μ l of 10% sodium dodecyl sulfate (SDS) (BioRad). The polypeptides were immediately reduced by addition of 5 μ l of 2-mercaptoethanol (Matheson, Coleman and Bell) and were then alkylated in the dark at room temperature for 1 h by the addition of two chemical equivalents of iodoacetamide (Sigma). The polypeptides were then separated from unreacted label and salts by P-4 (Biorad) chromatography with a 0.5-cm-diameter, 7-ml column and 0.1 M NH_4HCO_3 -0.1% SDS as the eluting solvent. The first radioactive peak was pooled and lyophilized.

Lyophilized polypeptides were suspended in 2 M urea (Mallinckrodt) and 0.02% bromophenol blue (Baker, analyzed reagent), incubated for 1 min in a boiling-water bath, and analyzed by discontinuous SDS-polyacrylamide gel electrophoresis as described by Laemmli (13), with several modifications (9). After electrophoresis, gels were fixed in 25% isopropanol (Baker) and 10% acetic acid (Baker), dried for 2 h, and exposed for screen-intensified autoradiography. Individual bands were excised from the gel, dried further overnight under high vacuum, and rehydrated with a solution of trypsin (Calbiochem, chymotrypsin free) as described (9). After lyophilization of the supernatants and redigestion to completion with trypsin, phosphopeptides were analyzed on 0.1-mm-thick thin-layer cellulose plates (Brinkmann). Thin-layer electrophoresis in the first dimension was carried out at 14°C for 2 h at 450 V in 8% acetic acid-2% formic acid (Baker) (pH 2). After drying, plates

were subjected to ascending chromatography at a 90° angle to electrophoresis in 1-butanol-pyridine-acetic acid-water at the ratio of 15:9:3:9 (vol/vol). After drying, the plates were exposed for screen-intensified autoradiography. Butanol and pyridine were purchased from Baker. For phosphoamino acid analysis, lyophilized polypeptides were precipitated in the presence of 10 μ g of lysozyme carrier protein (Sigma) by ice-cold 10% trichloroacetic acid (Baker). After the precipitate was collected by centrifugation, it was washed twice in absolute ethanol and then dissolved in 6 M HCl (Baker, analyzed reagent). The reaction vessel (Pasteur pipette) for hydrolysis was sealed under vacuum, and hydrolysis was carried out for 2 h at 100°C. The samples were lyophilized and then resuspended in 20 μ l of water, and portions were analyzed by two different separation procedures as outlined in the legend to Fig. 4. Phosphoserine and phosphothreonine were purchased from Calbiochem, and phosphotyrosine was synthesized as described (20, 23). Briefly, O⁴-phosphotyrosine was made by heating 1.4 g of L-tyrosine with 4.1 ml of 85% (wt/vol) phosphoric acid and 4.2 g of phosphoric acid anhydride to 100°C for 72 h. Phosphotyrosine was separated from tyrosine and inorganic phosphate by Dowex-50 column chromatography and collected by ethanol precipitation and filtration. The purity was assessed by thin-layer chromatography and by sensitivity to alkaline phosphatase.

HBcAg and GSHcAg particles were isolated from infected human and ground squirrel livers, respectively, by two steps of discontinuous CsCl density equilibrium centrifugation and a single step of CsCl rate-zonal sedimentation. After the first step of purification, both DNA polymerase and protein kinase activities were found at a density of 1.33 to 1.34 g/ml within the enzyme-linked immunosorbent assay positive peak (Fig. 1a and b). By the final step of purification (Fig. 1c and d), these core-associated activities separated so that polymerase was found associated with heavy core at higher density (1.36 g/ml) and protein kinase was found associated mainly with light core particles at slightly lower density (1.31 g/ml). These activities roughly correspond to the absorbancy profile of HBcAg in Fig. 1c, which clearly shows the presence of two peaks in the enzyme-linked immunosorbent assay positive fractions. GSHV heavy cores were not detected by absorbancy at the expected density (1.36 g/ml). However, the potent endogenous DNA polymerase activity revealed their presence at the same density as HBV heavy core particles. SDS-polyacrylamide gel electrophoresis of the phosphorylated products after the first step and after the final step of purification showed that the major core polypeptide in each case was the

FIG. 1. Discontinuous CsCl density equilibrium gradients of HBcAg and GSHcAg particles. Gradients were prepared and centrifuged as described in the text. Fraction densities were derived from refractive index determinations (●). The absorbancy curves were obtained by measurements at 280 nm on a Zeiss PM2 spectrophotometer (○). For antigen detection by enzyme-linked immunosorbent assays, the immunoglobulin G fractions of sera from HBcAg- or GSHcAg-positive carriers were used to coat flat-bottom microtiter plates (Dynatech) and for conjugation with horseradish peroxidase as described (3, 16) (—). DNA polymerase and protein kinase assays were carried out as described (1). Briefly, 50- μ l samples of each fraction were diluted to 0.5 ml, and the purified cores were pelleted overnight at 24,000 rpm in a type 25 rotor. For the polymerase reaction, 5 μ Ci each of [3 H]dCTP and [3 H]dGTP were added with cold nucleoside triphosphates and the polymerase reaction mixture to each of the pellets, and the samples were incubated for 3 h at 37°C. After termination of the reaction with EDTA, the samples were trichloroacetic acid precipitated and counted (vertical bars). Separate samples from each gradient were pelleted and assayed for protein kinase activity by the addition of 5 μ Ci of [γ - 32 P]ATP in protein kinase buffer to each pellet, followed by incubation at 37°C for 1 h. The reaction was terminated by the addition of SDS, and the fractions were trichloroacetic acid precipitated and finally assayed by counting (□). (a) HBcAg after single-step CsCl density equilibrium centrifugation; (b) GSHcAg after single-step CsCl density equilibrium centrifugation; (c) HBcAg after final step of CsCl density equilibrium centrifugation; (d) GSHcAg after final step of CsCl density equilibrium centrifugation.

dominant phosphate acceptor, indicating that the same protein kinase activity was being followed throughout purification (data not shown). These results suggest that protein kinase activity is associated mainly with polymerase-positive cores early on in the purification scheme (Fig. 1a and b) and that this may reflect a like association of these activities in the polymerase-positive core particles of infected liver. The shift of kinase activity from association with polymerase-positive to polymerase-negative cores during purification (Fig. 1c and d) may be a conse-

quence of purification and is consistent with recent documentation that core particles exposed to CsCl density gradients undergo morphological disintegration accompanied by conversion from core to e antigenic activity (18). These alterations might also include the loss of DNA from the majority of core particles during purification, and may include other undocumented changes. The finding of protein kinase activity in polymerase-negative cores during purification, however, also suggests that this activity may be present in multiple core subpopula-

tions in infected liver. It should also be noted that the quantities of tissues used for HBcAg and GSHcAg purification were not the same, nor was the titer of core particles in each starting sample known with certainty, indicating that the magnitude of core-associated activities in HBcAg and GSHcAg gradient profiles cannot be directly compared.

Phosphorylated polypeptides of HBcAg and GSHcAg were reduced, alkylated, and analyzed by SDS-polyacrylamide gel electrophoresis as shown in Fig. 2 and 3. Several lines of evidence suggest that the major core polypeptide of each virus contained most of the accepted phosphate, even though their mobilities were faster here than in a comparison study (8). The major core polypeptide of GSHcAg consistently migrated slightly faster on polyacrylamide gels than its HBcAg counterpart. This has also been the case when the core polypeptides were labeled with ^{125}I and similarly analyzed. Comparison of the cleaved polypeptides in the latter study revealed a larger difference in mobility between them than between the respective major components from which each was derived. Furthermore, a previous study (1) already demonstrated that even under conditions of immunoprecipitation with anticore, complete cleavage of the major polypeptide was not obtainable. Since antibody was not utilized here, and since labeled doublets between 18,000 and 22,000 daltons were not seen in any experiments, it is unlikely that p18 of

FIG. 2. SDS-polyacrylamide gel electrophoresis of protein kinase-labeled GSHcAg and HBcAg core polypeptides. (a) ^{32}P -labeled GSHcAg polypeptides; (b) ^{32}P -labeled HBcAg polypeptides.

GSHcAg	HBcAg
	>100
	>100
	68
	53
	48
	40
	34.5
	30.5
28	27.5
26	
17.5*	18*
15.5	
14	14
	12
11	11

FIG. 3. Molecular weight values ($\times 10^3$) of phosphorylated core polypeptides. Purified HBcAg or GSHcAg particles were pelleted overnight, and polypeptides were labeled by the associated protein kinase activity. Reduced and alkylated polypeptides were analyzed by SDS-polyacrylamide gel electrophoresis and detected by autoradiography of the dried gel. Asterisks next to p17.5 of GSHcAg and p18 of HBcAg indicate that these are the major labeled components of each profile.

HBcAg and p17.5 of GSHcAg in Fig. 2 are actually the cleaved products of the major polypeptides.

The results in Fig. 2 and 3 indicate a number of minor phosphorylated polypeptides higher and lower in molecular weight than the major labeled species. Furthermore, the number and size of these minor labeled components vary considerably in the core particles of each virus. Examination of phosphorylated tryptic peptides in each case revealed considerable homology in the sites of phosphorylation among the major polypeptide of human and ground squirrel core antigens (Fig. 4). Differences exist, however, in the extent to which these shared tryptic peptides are phosphorylated. The sites of phosphorylation in the polypeptides of various molecular weights within each virus core were exactly the same as the pattern seen in the major phosphorylated polypeptide (Fig. 4c; data for GSHcAg not shown). This suggests that HBcAg and GSHcAg share many sites of phosphorylation in the primary sequence of their major core polypeptide. All of the major phosphorylated tryptic peptides in the human core phosphotryptic peptide map are shared (Fig. 4d), and the differences noted on the composite were always minor on the autoradiograms. Each virus, then, may possess minor sites for phosphorylation. The striking pattern of conserved sites of phosphoryla-

tion independent of molecular weight in each virus might be the consequence of simple aggregation. However, tryptic peptide mapping of ^{125}I -labeled core polypeptides of GSHcAg and HBcAg, which were prepared and analyzed in a manner identical to that described above, showed that not all of these high-molecular-weight polypeptides were present as a consequence of aggregation (8). Indeed, most of the phosphorylated polypeptides of GSHcAg and HBcAg represent a subset of those labeled by ^{125}I .

The amino acid residues accepting phosphate during the *in vitro* protein kinase reaction were characterized on thin layers by using two separation systems (Fig. 5). Intact cores labeled by the kinase reaction were reduced and alkylated, trichloroacetic acid precipitated, and finally hydrolyzed. The major phosphorylated residue in

both viruses was serine, and the minor acceptor was threonine. No phosphotyrosine was detected in either virus core under any circumstances. When the three major phosphorylated tryptic peptides in Fig. 4a were individually hydrolyzed after separation by peptide mapping, each of them was found to be phosphorylated only at serine (data not shown).

The experiments herein describe the presence of a protein kinase activity associated with the liver-derived core particles of GSHV and demonstrate the relationship between this activity and that recently discovered in HBV cores (1). Both activities are associated with the DNA polymerase-positive heavy core fractions on CsCl gradients (which may convert to light cores during purification), phosphorylate mainly the major polypeptide of core particles as well as a number of minor polypeptides, and result in

FIG. 4. Tryptic phosphopeptides of HBcAg and GSHcAg polypeptides. The origin is located at the bottom right-hand corner of each plate. Thin-layer electrophoresis in the first dimension is from right to left, and ascending chromatography in the second dimension is from the bottom to the top of each autoradiogram. (a) Major polypeptide (p18) of HBcAg; (b) minor high-molecular-weight polypeptide (p40) of HBcAg; (c) major polypeptide (p17.5) of GSHcAg; (d) composite of the HBcAg (a) and GSHcAg (c) major polypeptides. Some of the minor spots drawn on the composite might not easily be seen on the autoradiograms.

FIG. 5. Phosphorylated amino acids of HBcAg and GSHcAg core particles. (a, b) Phosphorylated core polypeptides, partially hydrolyzed, were analyzed by thin-layer electrophoresis at pH 3.5 for 45 min at 1,000 V with acetic acid-pyridine-water, 50:5:945 (vol/vol; 10). Samples were spotted near the cathode (near bottom of autoradiogram), and electrophoresis was carried out at 14°C from bottom to top. (c, d) Phosphorylated, hydrolyzed samples were spotted near the cathode (bottom right-hand corner) and electrophoresed for 2 h at 750 V with acetic acid-formic acid-water, 4:1:50 (vol/vol; pH 2.0). Ascending chromatography was carried out in the second dimension (from bottom to top) with isobutyric acid-0.5 M NH_4OH , 5:3 (10). Phosphorylated serine, threonine, and tyrosine were analyzed together with the labeled hydrolysate and detected by ninhydrin staining. Radiolabeled amino acids were detected by screen-intensified autoradiography. (a, c) HBcAg; (b, d) GSHcAg.

phosphorylation of serine (major) and threonine (minor) residues within conserved regions of the core antigen polypeptide sequence. Despite differences seen in the phosphorylated polypeptide profile of each virus core with regard to the number and size of minor labeled components, the activities and acceptors share many similarities.

The characteristics of HBV and GSHV protein kinase activities are shared with any other animal viruses. The major phosphorylated components, in this case the respective major core polypeptide, clearly play an important structural role in the nucleocapsids of HBV and GSHV. However, any additional properties that may be conferred upon hepadna virus core polypeptides is not yet clear. The phosphorylation of a subset of nucleocapsid structural proteins has been recently described in vesicular stomatitis virus, and an increase in phosphorylation of the NS protein, a minor nucleocapsid component which is the major phosphorylated product of the kinase reaction, seems to be correlated with an increase in the rate of transcription in the vesicular stomatitis virus ribonucleoprotein core complex (12; S. U. Emerson, submitted for publication). The N-protein of rabies virus, also a nucleocapsid structural protein, is also found to be a phosphoprotein, and has been postulated to be an inactive transcriptase (proenzyme) which is activated by phosphorylation or proteolytic cleavage or both, after uncoating (28).

The fact that a large number of enveloped viruses possessing a protein kinase activity often require a detergent such as Nonidet P-40 (which dissolves the virus envelope) for expression (1, 30) may be analogous to a requirement for virus uncoating in vivo, which would permit access to the nucleocapsid of cellular nucleoside triphosphates. In rabies virus, serine is the major amino acid and threonine is the minor amino acid phosphorylated. Furthermore, the localization of the phosphorylated amino acids at one end of the N-protein suggests that nucleocapsid assembly occurs before phosphorylation. The small number of major phosphorylation sites in HBV and GSHV core particles is consistent with this conclusion, and further studies may localize the phosphorylated amino acids to a small portion of the core polypeptide primary sequence. The presence of shared tryptic peptides in HBV and GSHV core polypeptides also suggests that the structure and orientation of these components in the intact cores of each virus are similar. Differences may exist in that the extent of phosphorylation differs within some of these conserved sites. The finding of an RNA-dependent transcriptase in vesicular stomatitis virus activated by phosphorylation and an RNA-dependent DNA polymerase in avian myeloblastosis virus

activated by phosphorylation (32) suggests that one of the consequences of phosphorylation may be an alteration in the endogenous DNA polymerase activity of hepatitis viruses. Alternatively, phosphorylation may be coupled to the cleavage of the core antigen polypeptide recently shown to occur in immunoprecipitates with anticore (1). In this context it should be noted that HBcAg-associated e antigen has been detected in the presence of SDS after treatment of core particles with pronase (5, 29).

An important relationship exists between the state of nucleocapsid polypeptide phosphorylation and nucleic acid binding with a number of viruses. This property need not be considered mutually exclusive from the changes in enzymatic properties discussed above, since the regulation of transcription or reverse transcription may be partially dependent upon DNA or RNA binding. The p12^{gag} protein of Rauscher murine leukemia virus, which is a structural protein residing in the nucleocapsid of this virus, is heterogeneously phosphorylated at serine residues (25, 26). Phosphorylation of this protein has been shown to be inversely proportional to its RNA genome binding capability. A phosphorylated subpopulation of polyoma VP1 has also been shown to bind tightly to its nucleoprotein core (4). In each of these cases, regulation of transcription by differentially phosphorylated subpopulations of structural nucleocapsid proteins has been suggested. Since the proposed amino acid sequence of HBV core polypeptide suggests a protein having a carboxy-terminal series of basic residues (19, 31), it is possible that these residues, like those of basic histones, may be involved in DNA binding. Differential phosphorylation of core polypeptide(s), like that of histones, may provide one means by which virus gene expression may be regulated. Further characterization of the major core polypeptide with respect to DNA binding and heterogeneity in phosphorylation will clarify the relationship among these parameters.

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LITERATURE CITED

1. Albin, C., and W. S. Robinson. 1980. Protein kinase activity in hepatitis B virus. *J. Virol.* 34:297-302.
2. Almeida, J. D., D. Rubenstein, and E. J. Stott. 1971. New antigen-antibody system in Australia-antigen-positive hepatitis. *Lancet* ii:1225-1227.
3. Avrameas, S., and T. Ternynck. 1971. Peroxidase-labeled antibody and Fab conjugates with enhanced intracellular penetration. *Immunochemistry* 8:1175-1179.
4. Bolen, J. B., D. G. Anders, J. Trempey, and R. A. Consigli. 1981. Differences in the subpopulation of the structural

- proteins of polyoma virions and capsids: biological functions of the multiple VP₁ species. *J. Virol.* 37:80–91.
5. Budkowska, A., B. Kalinowska, and A. Nowoslawski. 1979. Identification of two HBcAg subspecificities revealed by chemical treatment and enzymatic digestion of liver derived HBcAg. *J. Immunol.* 123:1415–1416.
 6. Dane, D. S., C. H. Cameron, and M. Briggs. 1970. Virus-like particles in serum of patients with Australia-antigen associated hepatitis. *Lancet* i:695–698.
 7. Feitelson, M. A., P. L. Marion, and W. S. Robinson. 1981. Antigenic and structural relationships of the surface antigens of hepatitis B virus, ground squirrel hepatitis virus, and woodchuck hepatitis virus. *J. Virol.* 39:447–454.
 8. Feitelson, M. A., P. L. Marion, and W. S. Robinson. 1982. Core particles of hepatitis B virus and ground squirrel hepatitis virus. I. Relationship between hepatitis B core antigen- and ground squirrel hepatitis core antigen-associated polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and tryptic peptide mapping. *J. Virol.* 43:687–696.
 9. Gerlich, W. S., M. A. Feitelson, P. L. Marion, and W. S. Robinson. 1980. Structural relationships between the surface antigens of ground squirrel hepatitis virus and human hepatitis B virus. *J. Virol.* 36:787–795.
 10. Hunter, T., and B. M. Sefton. 1980. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc. Natl. Acad. Sci. U.S.A.* 77:1311–1315.
 11. Kaplan, P. M., R. L. Greenman, J. L. Gerin, R. H. Purcell, and W. S. Robinson. 1973. DNA polymerase associated with human hepatitis B antigen. *J. Virol.* 12:995–1005.
 12. Kingsford, L., and S. U. Emerson. 1980. Transcriptional activities of different phosphorylated species of NS protein purified from vesicular stomatitis virions and cytoplasm of infected cells. *J. Virol.* 33:1097–1105.
 13. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T₄. *Nature (London)* 227:680–685.
 14. Landers, T. A., H. B. Greenberg, and W. S. Robinson. 1977. Structure of hepatitis B Dane particle DNA and nature of the endogenous DNA polymerase reaction. *J. Virol.* 23:368–376.
 15. Marion, P. L., L. S. Oshiro, D. C. Regnery, G. H. Scullard, and W. S. Robinson. 1980. A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. *Proc. Natl. Acad. Sci. U.S.A.* 77:2941–2945.
 16. Nakane, P. K., and A. Kawaoi. 1974. Peroxidase-labeled antibody. A new method of conjugation. *J. Histochem. Cytochem.* 22:1084–1091.
 17. Neurath, A. R., and N. Strick. 1979. Association of hepatitis B e antigen (HBcAg) determinants with the core of Dane particles. *J. Gen. Virol.* 42:645–649.
 18. Ohori, H., M. Yamaki, S. Onodera, E. Yamada, and N. Ishida. 1980. Antigenic conversion from HBcAg to HBsAg by degradation of hepatitis B core particles. *Intervirology* 13:74–82.
 19. Pasek, M., T. Goto, W. Gilbert, B. Zink, H. Schaller, P. MacKay, G. Leadbetter, and K. Murray. 1979. Hepatitis B virus genes and their expression in *E. coli*. *Nature (London)* 282:575–579.
 20. Plimmer, R. H. A. 1941. Esters of phosphoric acid. Phosphoryl hydroxylaminoacids. *Biochem. J.* 35:461–469.
 21. Robinson, W. S., D. A. Clayton, and R. L. Greenman. 1974. DNA of a human hepatitis B virus candidate. *J. Virol.* 14:384–391.
 22. Robinson, W. S., and R. L. Greenman. 1974. DNA polymerase in the core of the human hepatitis B virus candidate. *J. Virol.* 13:1231–1236.
 - 22a. Robinson, W. S., P. L. Marion, M. A. Feitelson, and A. A. Siddiqui. 1981. The Hepadna virus group: hepatitis B and related viruses, p. 57–68. *In* W. Szmunes, H. J. Alter, and J. E. Maynard (ed.), *Proceedings of the International Symposium on Viral Hepatitis*. Franklin Institute Press, Philadelphia, Pa.
 23. Rothberg, P. G., T. J. R. Harris, A. Nomoto, and E. Wimmer. 1978. O⁴-(5'-Uridyl)tyrosine is the bond between the genome-linked protein and the RNA of poliovirus. *Proc. Natl. Acad. Sci. U.S.A.* 75:4868–4872.
 24. Sattler, F., and W. S. Robinson. 1979. Hepatitis B viral DNA molecules have cohesive ends. *J. Virol.* 32:226–233.
 25. Sen, A., C. J. Sherr, and G. J. Todaro. 1976. Specific binding of the type C viral core protein p12 with purified viral RNA. *Cell* 7:21–32.
 26. Sen, A., C. J. Sherr, and G. J. Todaro. 1977. Phosphorylation of murine type C viral p12 proteins regulates their extent of binding to the homologous viral RNA. *Cell* 10:489–496.
 27. Siddiqui, A., P. L. Marion, and W. S. Robinson. 1981. Ground squirrel hepatitis virus DNA: molecular cloning and comparison with hepatitis B virus DNA. *J. Virol.* 28:393–397.
 28. Sokol, F., and H. F. Clark. 1973. Phosphoproteins, structural components of rhabdoviruses. *Virology* 52:246–263.
 29. Takahashi, K., Y. Akahane, T. Gotanda, T. Mishiro, M. Imai, Y. Miyakawa, and M. Mayumi. 1979. Demonstration of hepatitis B e antigen in the core of Dane particles. *J. Immunol.* 122:275–279.
 30. Tan, K. B. 1975. Comparative study of the protein kinase associated with animal viruses. *Virology* 64:566–570.
 31. Tiollais, P., P. Charnay, and G. N. Vyas. 1981. Biology of hepatitis B virus. *Science* 213:406–411.
 32. Tsiapalis, C. M. 1977. Chemical modification of DNA polymerase phosphoprotein from avian myeloblastosis virus. *Nature (London)* 266:27–31.