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Hepatitis B Virus Nucleocapsid Particles Do Not Cross the Hepatocyte Nuclear Membrane in Transgenic Mice†

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Transgenic mice that express the hepatitis B virus core protein were used to examine factors that influence the intracellular localization of nucleocapsid particles in the primary hepatocyte in vivo. In this model, viral nucleocapsid particles are strictly localized to the nucleus of the hepatocyte except when the nuclear membrane dissolves during cell division, at which time they enter the cytoplasm. The cytoplasmic nucleocapsid particles do not reenter the nucleus, however, when the nuclear membrane re-forms after cell division. The data support the notion that nucleocapsid particles can form de novo within the nucleus, and they suggest that preformed nucleocapsid particles cannot be transported across the intact nuclear membrane in either direction. The results imply that nucleocapsid disassembly is probably required for entry of the hepadnaviral genome into the nucleus, and they question the role of the intranuclear viral nucleocapsid particle during the viral life cycle.

The hepatitis B virus (HBV) nucleocapsid is an icosahedral 28-nm particle that consists of approximately 180 repeating subunits of a 21-kDa protein (core protein) that contains carboxy-terminal packaging (2) and nuclear localization signals (6, 23), and it expresses the hepatitis B core antigen (HBcAg). During HBV infection, the nucleocapsid packages the viral pregenomic RNA and polymerase protein into a structure that facilitates viral replication and interacts with viral envelope proteins to form infectious virions that are secreted by the cell (8). Additionally, the nucleocapsid is thought to deliver the viral genome to the nuclei of infected cells, both to initiate and to amplify viral replication during the viral life cycle (8). It is not known whether the nucleocapsid particle actually crosses the nuclear membrane and enters the nucleus during this process or whether it is arrested at the nuclear membrane, where it disassembles before entry of the viral genome can occur.

Since HBV nucleocapsid particles have been shown to be present in both the nucleus and the cytoplasm of HBV-infected hepatocytes (12, 16, 17), it is possible that intact nucleocapsid particles are able to move in one or both directions across the nuclear membrane. Such movement would presumably require an active or facilitated transport process, since the diameter of the nucleocapsid particle (28 nm) greatly exceeds the estimated diameter (9 nm) of nuclear pores (7). Indeed, gold particles up to 26 nm in diameter that are coated with nuclear targeting signals have been shown to enter the nuclei of *Xenopus* oocytes (5). Since nucleocapsid assembly from core protein monomer or dimers is a concentration-dependent process (20, 25, 27), it is also possible that nucleocapsid particles can form de novo on both sides of the nuclear membrane whenever the concentration threshold for particle assembly is reached. Importantly, HBV nucleocapsids in *Xenopus* oocytes that have been injected with synthetic mRNA encoding the HBV core protein are located exclusively in the cytoplasm (26), as are duck hepatitis B virus (DHBV) nucleocapsid particles in heavily infected duck hepatocytes (18, 22). These observations suggest that the nuclear membrane may not permit entry of preformed nucleocapsids into the nucleus and that the presence of nuclear core particles implies their de novo assembly within the nucleus rather than their transport across the nuclear membrane as intact particles.

In view of the importance of these events in the viral life cycle, the nature of the interaction between the HBV nucleocapsid particle and the nuclear membrane is a matter of considerable interest. We have used transgenic mice that express the HBV core protein to examine this question. In these animals, HBV nucleocapsid particles are located exclusively in the nucleus of both resting and cycling hepatocytes as long as the nuclear membrane is intact, but they are released into the cytoplasm when the nuclear membrane disintegrates during mitosis, and they do not reenter the nucleus when it re-forms.

These observations demonstrate that HBV nucleocapsid particles cannot cross the nuclear membrane in either direction in this model system. If the same is true for HBV genome-containing nucleocapsids, the current observations would suggest that entry of the viral genome into the nucleus during natural viral infection requires nucleocapsid particle disassembly on the cytoplasmic side of the nuclear membrane. Additionally, these observations force reevaluation of the biological significance of intranuclear nucleocapsids during the HBV life cycle, if they cannot leave the nucleus once they have formed.

MATERIALS AND METHODS

HBV transgenic mice. Lineage MUP-core 50 (MC50) (official designation, Tg[MUP,HBVcore]Chi50) was produced by microinjection of a subgenomic fragment of the HBV genome containing approximately 0.9 kb (nucleotides [nt] 1887 to 2800) of the 5′ core coding region downstream the mouse major urinary promoter (10) and upstream of the simian virus 40 polyadenylation site (10) into (C57BL/6 × SJL)F_2 embryos.

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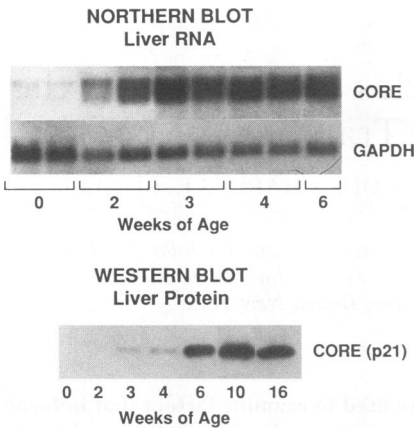

FIG. 1. Developmental regulation of HBV core expression in lineage MC50. (Top) Northern blot analysis of 20 μg of total liver RNA hybridized with HBV- and GAPDH-specific probes. The results obtained from two male transgenic mice at each time point are indicated. (Bottom) Western blot analysis of 100 μg of total liver protein from transgenic males probed with HBc/eAg-specific antibodies as described in the text.

The lineage was expanded by repetitive backcrossing against the C57BL/6 parental strain. The MC50 founder and transgenic progeny were identified by detection of HBV core-specific DNA sequences in tail DNA, using PCR with HBV core region-specific primers (antisense [5′-CTAACATTGAG ATTCCCGA, nt 2336 to 2321] and sense [5′-CCTCACCATA CAGCACTCAGGCAA, nt 2050 to 2073). Structural analysis of the integrated transgene reveals that at least one complete, uninterrupted copy is integrated at a single site in the mouse genome (not shown).

Analysis of HBV gene expression. (i) RNA analysis. Specific HBV and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was assessed by Northern (RNA) blot hybridization of 20 μg of total RNA prepared from liver, kidney, pancreas, spleen, thymus, heart, lung, brain, stomach, intestine, testis, skeletal muscle, thyroid, adrenal glands, skin, and bone marrow as previously described (9). Expression of the transgene is limited to the liver (not shown).

(ii) Protein analysis. Serum and organ homogenates were prepared as described previously (15). Total soluble protein was determined by Coomassie blue G-250 binding (Bio-Rad). HBc/eAg was measured by solid-phase radioimmunoassay (AUSRIA-II; Abbott), by enzyme-linked immunosorbent assays, and by Western blotting (immunoblotting) as previously described (3, 15).

(iii) Immunohistochemical analysis. The intrahepatic distribution of HBc/eAg and proliferating cell nuclear antigen (PCNA) was assessed by the labeled avidin-biotin detection procedure (3). Paraffin-embedded sections in phosphate-buffered saline (PBS; pH 7.4) were treated for 10 min at 37°C with 3% hydrogen peroxide and washed with PBS. Sections were blocked with normal goat serum, and a rabbit anti-HBc/eAg or mouse anti-PCNA (Dako, Carpinteria, Calif.) primary antiserum was applied at a 1:100 dilution for 60 min at 37°C (HBcAg) or overnight at 4°C (PCNA). After a wash with PBS, a secondary antiserum of biotin-conjugated goat anti-rabbit immunoglobulin G $F(ab')_2$ (HBc/eAg) or biotin-conjugated goat anti-mouse immunoglobulin G $F(ab')_2$ (Sigma Corp., St. Louis, Mo.) was applied at a 1:100 dilution for 30 min at 37°C. The antibody-coated slides were washed with PBS, treated with streptavidin-horseradish peroxidase conjugate (Extravidin; Sigma) at a 1:600 dilution for 30 min at 37°C, stained with 3-amino-9-ethyl carbazole (Shandon-Lipshaw, Pittsburgh, Pa.) (HBcAg) or 3,3′-diaminobenzidine tetrahydrochloride (Sigma) (PCNA), and counterstained with Mayer's hematoxylin (HBcAg) or nuclear fast red (PCNA) before mounting.

Electron microscopy. Thin liver sections were fixed overnight at 4°C in 4% paraformaldehyde–0.1% glutaraldehyde in PBS. Sections were then postfixed in 1% OsO_4 in cacodylate buffer (pH 7.4) for 1 h at room temperature, dehydrated in gradient ethanol, and embedded in epoxy resin (TAAB 812; Emmer Green, Reading, England). Sections were cut on an LKB Ultratome III, mounted on copper grids, stained in uranyl acetate and lead citrate, and viewed with a Hitachi HU 12-A electron microscope.

PH. Eight- to ten-week-old females were anesthetized and subjected to 50 or 70% partial hepatectomy (PH) as described previously (11). At 36, 48, 64, and 72 h and at 7, 10, 14, and 21 days post-PH, mice were anesthetized and euthanized. RNA and protein analysis of pre- and post-PH livers was carried out as described above.

RESULTS

Analysis of transgene expression. Lineage MC50 expresses the HBV core open reading frame under the transcriptional control of the mouse major urinary protein promoter. Expression from this promoter has been previously demonstrated to be developmentally regulated at the RNA and protein levels (1). As shown in Fig. 1, trace amounts of core RNA and protein are present in the liver at birth, and they increase progressively, reaching plateaus by 3 and 6 weeks of age, respectively. The 3-week lag between the RNA and HBcAg kinetics suggests that the accumulation rate of HBcAg in the liver of these mice is rather slow. Immunochemical analysis of liver homogenates from 8-week-old mice revealed 1.05 ng of HBc/eAg per mg of total liver protein. In contrast, HBc/eAg was not detectable in the sera of these animals (not shown), compatible with the behavior of native core protein (8, 15).

Nuclear localization of HBV core protein. By 6 weeks of age, nearly 100% of hepatocyte nuclei stain strongly for HBc/eAg, while the cytoplasm is entirely negative (i.e., N^+ C^- phenotype) (Fig. 2A). At the electron microscopic level, the hepatocyte nuclei contain abundant 25- to 30-nm particles that display the characteristic ultrastructural features (Fig. 2B) of HBV nucleocapsids in infected human liver (19). In contrast, these structures are absent from the HBcAg-negative cytoplasm in these cells and from HBcAg-negative hepatocyte nuclei from nontransgenic control mice (not shown).

Since the antibody used in the immunohistochemical studies recognizes both particulate and soluble forms of the nucleocapsid protein (21), the data indicate that neither form of HBcAg accumulates to a detectable level in the cytoplasmic compartment of the hepatocyte in these mice, while it does in the nucleus. This finding suggests that the rate at which HBcAg is translocated into the nucleus is greater than its net rate of accumulation (synthesis minus degradation) in the cytoplasm. We cannot determine whether a low rate of HBcAg synthesis or a high rate of HBcAg degradation in the cytoplasm is responsible for this observation at this time. The data also suggest that HBcAg is quite stable once it reaches the nuclear compartment, suggesting that it may be degraded less rapidly in the nucleus than in the cytoplasm. The data also suggest that nucleocapsid particle formation occurs de novo in the nucleus and that nucleocapsid particles do not enter the cytoplasm from the intact nucleus once they have formed, as one would

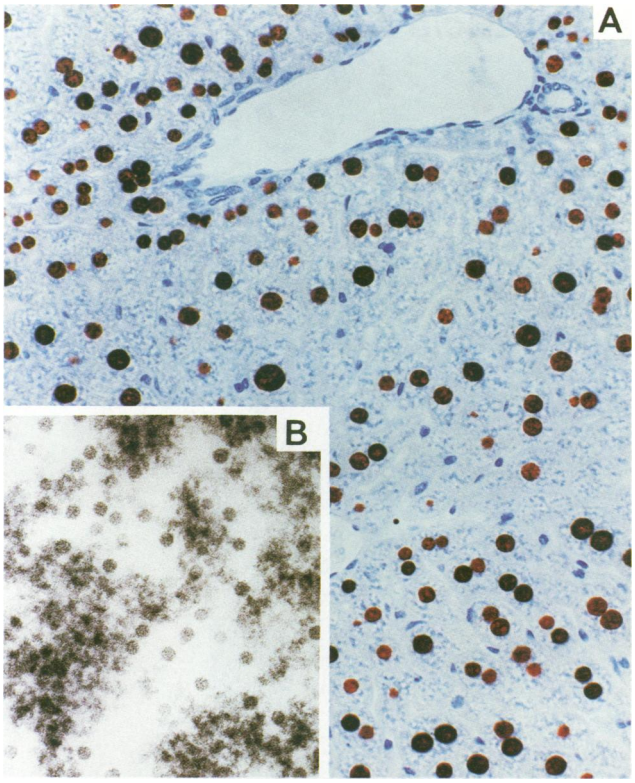

FIG. 2. Nuclear localization of HBV core protein in lineage MC50. (A) Immunohistochemical analysis of a 6-week-old male shows that HBc/eAg is exclusively localized in the hepatocyte nuclei. (Immunoperoxidase stain for HBc/eAg; original magnification, ×200.) (B) Electron microscopic analysis of the same nuclei reveals abundant 25- to 30-nm particles. (Original magnification, ×30,000.)

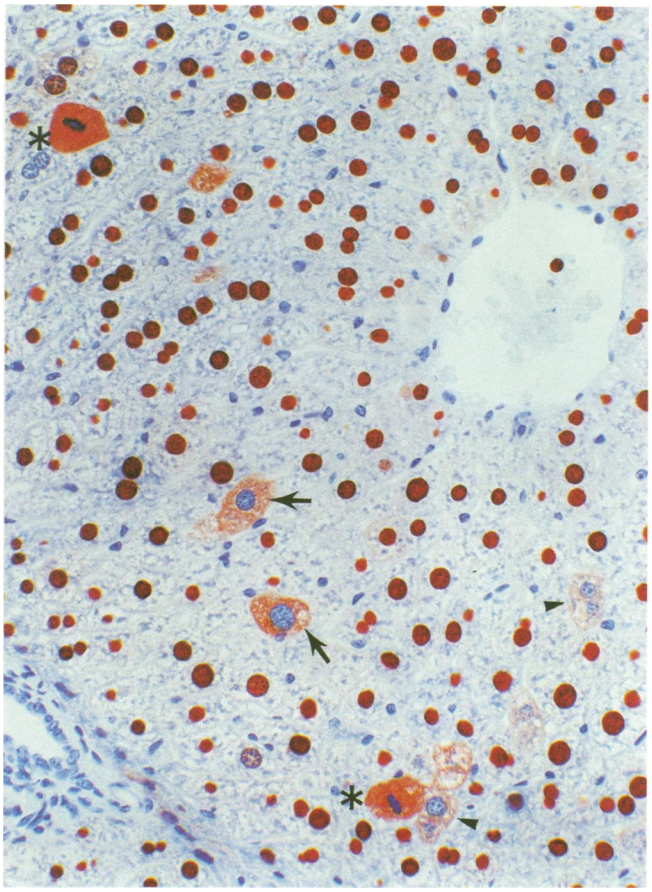

FIG. 3. Cytoplasmic localization of HBV core protein. Hepatic HBc/eAg immunohistochemical analysis of a 12-week-old male shows the presence of mitotically active hepatocytes (asterisks) and hepatocytes that display HBc/eAg-positive cytoplasm and intact negative nuclei (arrows). These hepatocytes were frequently binucleate or occurred in pairs (arrowheads). (Immunoperoxidase stain for HBc/eAg; original magnification, ×200.)

expect if the nuclear HBcAg particles are effectively excluded from the cytoplasmic compartment by the nuclear membrane.

These observations are most compatible with the notion that core monomer and dimers are efficiently transported from the large cytoplasmic compartment into the smaller nuclear compartment, where they reach the concentration threshold required for nucleocapsid particle assembly to occur. While it is formally possible that nucleocapsid particles might also assemble in the cytoplasm at low core monomer and dimer concentrations and be transported rapidly, as particles, across the nuclear membrane into the nucleus, where they subsequently accumulate to detectable levels, this hypothesis is excluded by the following observations.

Cytoplasmic localization of HBV core protein. Whereas HBc/eAg is localized exclusively in the nucleus (N^+ C^-) in young mice, it is occasionally detectable in the cytoplasm of rare hepatocytes in older animals (Fig. 3). Two important features of this phenotype are noteworthy. First, it is characterized by the presence of mitotically active hepatocytes, the cytoplasm of which is always HBc/eAg positive (Fig. 3, asterisks). Second, HBc/eAg is never detected simultaneously in the nuclei and the cytoplasm of the same hepatocyte in these animals. Indeed, the nuclei of the hepatocytes that display cytoplasmic HBc/eAg are always negative for this antigen (N^- C^+) (Fig. 3, arrows). The closeness of the association between N^- C^+ hepatocytes and hepatocyte turnover suggests that N^- C^+ cells are in either a premitotic or postmitotic state that redistributes HBc/eAg within the cell.

HBV core protein is nuclear during all premitotic phases of the cell cycle. To test this hypothesis, we induced cell division in the livers of 8- to 9-week old MC50 transgenic mice by partial hepatectomy and monitored the intracellular distribution of HBc/eAg in the hepatocytes at different stages of the cell cycle. Using the expression of PCNA as a marker of cells in S phase (13), we observed many hepatocytes that were simultaneously PCNA positive (Fig. 4C) and HBc/eAg positive (Fig. 4D) 36 h after 70% PH, indicating that HBcAg is not transported out of the nucleus during S phase. This finding contrasts with a recent report that nuclear HBc/eAg is translocated into cytoplasm during S phase in transfected cell lines (24). The reasons for the discrepancy between the previous observations and our own are not immediately apparent, although the experimental systems are quite different from each other.

Not only is HBc/eAg localized to the hepatocyte nucleus during S phase in our animals, but it remains inside the nucleus through prophase, since by electron microscopy we observed nucleocapsid particles in the nucleus (Fig. 5A, arrows) but not in the cytoplasm of hepatocytes containing the characteristic ultrastructural features of prophase (condensed chromosomes and intact nuclear membrane) (14). These observations indi-

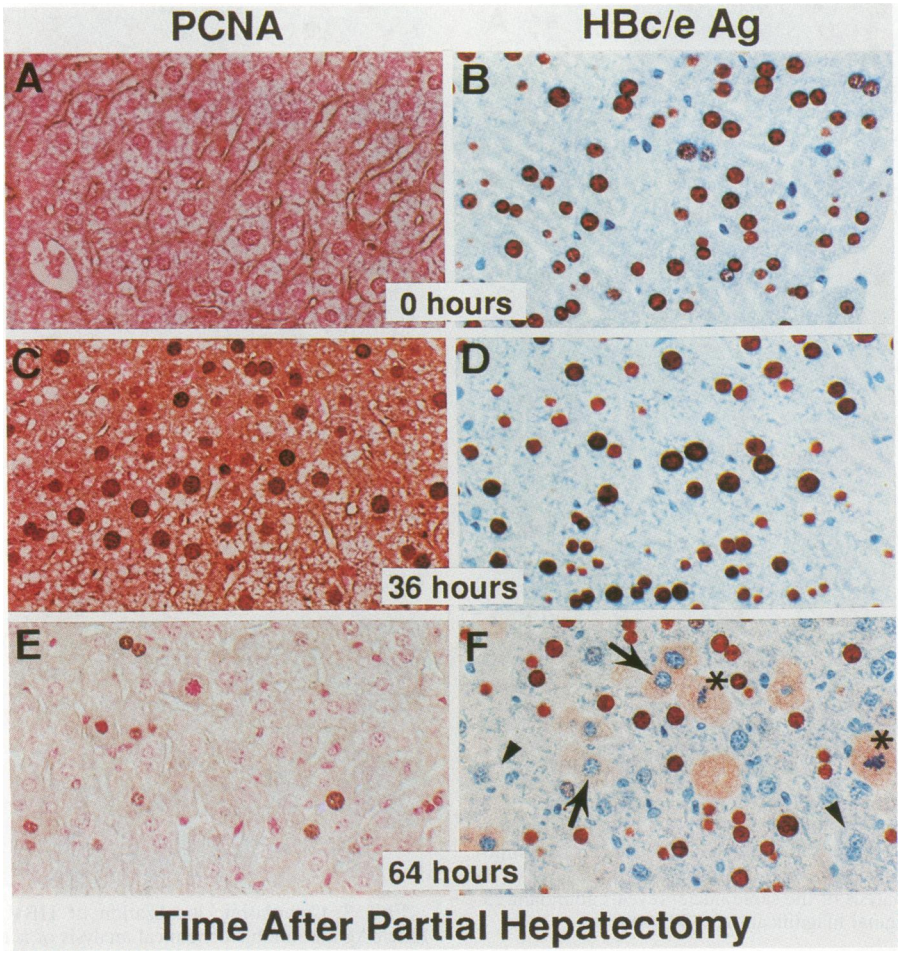

FIG. 4. Intracellular localization of HBV core protein at different stages of the cell cycle. Sequential liver biopsies obtained from 8- to 9-week-old transgenic females before and at different time points after PH, stained for the presence of PCNA (A, C, and E) and HBc/eAg (B, D, and F). (A and B) Resting liver; (C and D) liver harvested 36 h after 70% PH; (E and F) liver harvested 64 h after 50% PH. Note the mitotically active hepatocytes (asterisks), hepatocytes that display HBc/eAg-positive cytoplasm and intact negative nuclei (arrows), and completely HBc/eAg-negative cells (arrowheads) in panel F. (Immunoperoxidase stain for PCNA and HBc/eAg; original magnification, ×200.)

cate that HBV nucleocapsid particles remain in the nucleus throughout the cell cycle as long as the nuclear membrane is intact. The data also imply that the spontaneous appearance of cytoplasmic HBc/eAg in hepatocytes with HBc/eAg-negative nuclei, as we observed in older males from this lineage (Fig. 3), probably also reflects a postmitotic event. This hypothesis was addressed by monitoring the intracellular distribution of HBc/eAg at later time points following partial hepatectomy.

HBV core protein is cytoplasmic and particulate during and after mitosis. Sixty-four hours after 50% PH, most of the hepatocytes are PCNA negative (Fig. 4E), indicating that they have progressed beyond S phase, and many hepatocytes have entered mitosis and display cytoplasmic HBc/eAg (Fig. 4F) that presumably was released from the nucleus upon dissolution of the nuclear membrane. At the ultrastructural level, the cytoplasmic HBc/eAg was found to be particulate in the mitotic cells (Fig. 5B, arrows). In addition to the mitotic hepatocytes, many cells containing HBc/eAg-positive cytoplasm, and intact but HBc/eAg-negative nuclei are also present at this time point (Fig. 4F), reminiscent of the spontaneous association of the same phenotype with mitotic hepatocytes in older animals as described above (Fig. 3). It is germane that frequently these cells were binucleate or occurred in pairs (Fig. 3, arrowheads; Fig. 4F, upper arrow), suggesting that they had recently divided. The failure of these cytoplasmic nucleocapsid particles to reenter the nucleus, even though their cytoplasmic concentration is high enough for them to be detectable immunohistochemically and ultrastructurally, suggests that their transport is blocked, presumably at the level of the nuclear membrane. Alternative explanations for this observation include induced active transport of HBcAg particles out of the intact nucleus or accelerated HBcAg degradation in the nucleus coupled with accelerated net synthesis and particle formation in the cytoplasm following PH. Neither of these scenarios is compatible with the demonstrated presence of particulate HBcAg in the nucleus throughout S phase (Fig. 4D) and prophase (Fig. 5A) and its appearance in the cytoplasm immediately after nuclear membrane dissolution (Fig. 5B). The latter scenario is not compatible with the absence of evidence of accelerated HBcAg synthesis at the RNA or protein levels (see Fig. 7) following PH.

We also observed many completely HBc/eAg-negative cells in these livers (Fig. 4E, arrowheads), possibly reflecting dilu-

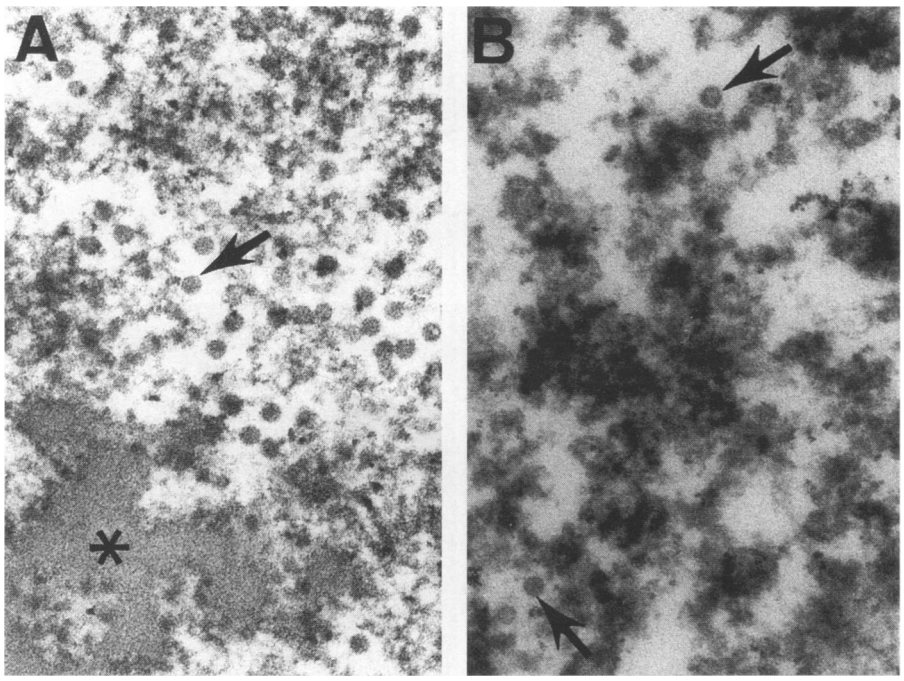

FIG. 5. Nucleocapsid particles are located in the nucleus during prophase and in the cytoplasm during mitosis. Characteristic 25- to 30-nm nucleocapsid particles (arrows) are detectable in the nuclei of prophase hepatocytes (A) containing condensed chromosomes (asterisk) and in the cytoplasm of M-phase hepatocytes (B). (Electron microscopy; original magnification, ×30,000.)

tion or destruction of HBc/eAg in the daughter cells after cell division (see below).

Reappearance of newly formed HBV core protein in the nucleus is a relatively slow process. Since nucleocapsid particle formation apparently does not occur in the cytoplasm in these animals, it is likely that the net rate of core monomer and dimer synthesis is equal to or lower than the rate at which HBc/eAg is transported out of the cytoplasm into the smaller nuclear compartment, where the threshold concentration needed for particle assembly (20) can be more easily attained. If this is correct, one would expect the accumulation rate of HBc/eAg anywhere in the hepatocyte to be quite low and that the antigen would appear first in the nucleus. As stated earlier, the 2- to 3-week lag between the rates at which core mRNA and core protein reach steady-state plateaus during neonatal life (Fig. 1) is compatible with this hypothesis.

To confirm this observation, we monitored the rate of reappearance of hepatocellular HBc/eAg in adult mice at different time points after 70% PH. In these mice, hepatocellular HBc/eAg becomes undetectable within 72 h (Fig. 6 and 7). Since there is no change in the hepatic steady-state content of nucleocapsid mRNA in these mice, we assume either that the loss of detectable HBc/eAg is due to its dilution in the two to three generations of daughter cells that develop after application of this strong mitogenic stimulus or that it is destroyed. Importantly, reappearance of HBc/eAg occurs very slowly in these animals (Fig. 6D to F and 7), and when it does reappear, it is again located in the nucleus. Notably, fewer than 50% of the hepatocytes displayed HBc/eAg-positive nuclei as late as 21 days after partial hepatectomy (Fig. 6F) despite the fact that the hepatic steady-state content of core mRNA remained at prehepatectomy levels at all time points studied (Fig. 7), again demonstrating that accumulation of nuclear HBc/eAg is a very slow process in this model system.

DISCUSSION

In this report, we have focused on the transport of HBcAg particles across the hepatocyte nuclear membrane under physiological conditions and during induced cell division in the living animal. Specifically, we have shown the following.

(i) It takes several weeks for HBcAg to become initially detectable in the hepatocyte during early life (Fig. 1), suggesting that its net accumulation rate (synthesis minus degradation) is quite slow in this model.

(ii) HBcAg is strictly nuclear and particulate in the resting hepatocyte (Fig. 2), suggesting that the rate of transport of HBcAg into the nucleus is faster than the rate at which it accumulates in the cytoplasm and that the particles do not move freely from nucleus to cytoplasm once they have formed.

(iii) HBcAg particles remain in the nucleus during S phase (Fig. 4D) and even during prophase (Fig. 5A), when the chromosomes have started to condense but before the nuclear membrane has started to dissolve.

(iv) HBcAg first becomes detectable in the cytoplasm of the hepatocyte during mitosis (asterisks in Fig. 3 and 4F), and it is particulate (Fig. 5B), suggesting that it is released into the cytoplasm only when the nuclear membrane dissolves.

(v) HBcAg is strictly cytoplasmic after the nucleus re-forms (arrows in Fig. 3 and 4F), indicating that it cannot move freely from the cytoplasm to the nucleus either and that it can survive in the cytoplasm, at least long enough to be detectable by this technique.

(vi) HBcAg subsequently becomes completely undetectable in the entire cell (Fig. 6C), presumably due to dilution (secondary to cell division) and/or to cytoplasmic degradation of the particles faster than the net rate of synthesis of new HBcAg protein in the cytoplasm.

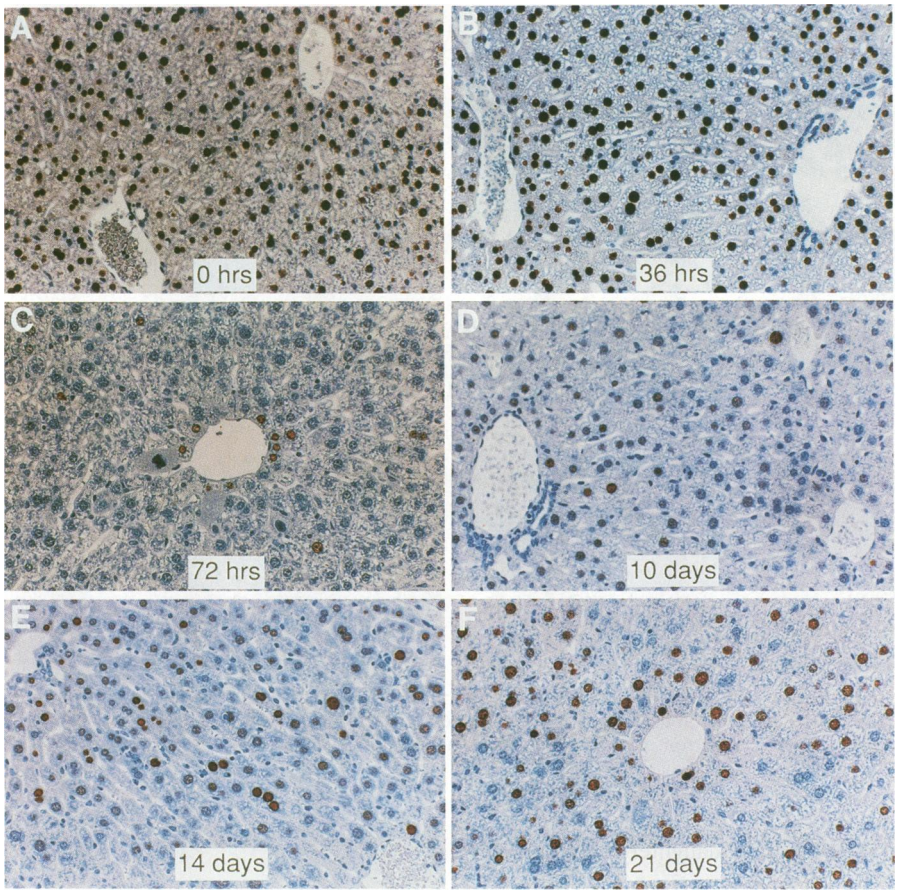

FIG. 6. Kinetics of loss and reappearance of nuclear HBc/eAg following 70% PH. Eight- to nine-week-old females were analyzed immunohistochemically for hepatic HBc/eAg content at different time points after 70% PH as indicated. Note the profound decrease of HBc/eAg immunoreactivity that occurs between 36 and 72 h and the prolonged duration required for the reaccumulation of immunohistochemically detectable HBc/eAg in the hepatocytes. (Immunoperoxidase stain for HBc/eAg; original magnification, ×200.)

(vii) It takes several weeks for HBcAg to reappear after PH (Fig. 6D to F), confirming that the rate of HBcAg protein accumulation is very slow in these cells.

On the basis of these findings, we conclude that the net rate of synthesis of HBcAg protein in the cytoplasm is less than its rate of transport into the nucleus in these mice, and thus the critical concentration threshold required for HBcAg particle assembly is reached only in the nuclei of these hepatocytes. Of course, this does not imply that core particle formation cannot occur in the cytoplasm if the net accumulation rate permits the assembly threshold to be reached in that compartment.

Collectively, the ultrastructural and immunohistochemical data strongly suggest that HBcAg is transported across the nuclear membrane as polypeptide monomers or dimers, where they accumulate until, as suggested by Seifer et al. for cytoplasmic core particle assembly (20), they reach a threshold concentration required for nucleocapsid particle assembly to occur. The data also suggest that these particles remain in the nucleus throughout the premitotic phase of the cell cycle, that they are released into the cytoplasm only during mitosis when the nuclear membrane dissolves, and that they cannot reenter the nucleus from the cytoplasm when the nuclear membrane re-forms, probably because of their particulate nature.

This concept is supported by observations for the DHBV system. It is well known that DHBV nucleocapsid particles are exclusively cytoplasmic during DHBV infection (18, 22), suggesting that the rate of DHBcAg particle formation in the cytoplasm is greater than the rate of monomer or dimer transport into the nucleus in that system and that the assembled cytoplasmic nucleocapsid particles cannot cross the intact nuclear membrane. This hypothesis is strengthened by the observation that mutations in the capsid gene of DHBV that preclude the assembly of nucleocapsid particles result in the accumulation of unassembled capsid antigen in the nuclei as well as the cytoplasm of infected cells (21a).

If the concept that the nuclear membrane serves as a barrier to nucleocapsid particle movement between the nuclear and cytoplasmic compartments is correct, and if it extends to viral genome-containing nucleocapsid particles during natural HBV infection, it carries at least two important implications for hepadnavirus biology. First, it suggests that nucleocapsid disassembly must occur, presumably at the nuclear membrane, to permit the viral genome to enter the nucleus during the first round or subsequent rounds of viral replication. Second, it questions the relevance of the intranuclear nucleocapsid particles for the viral life cycle. For example, if the intranuclear nucleocapsid particles are empty, their role in viral replication would be indirect, at best, and currently obscure. If, on the other hand, they do contain complete viral genomes, they should be inaccessible to the cellular transcriptional machinery

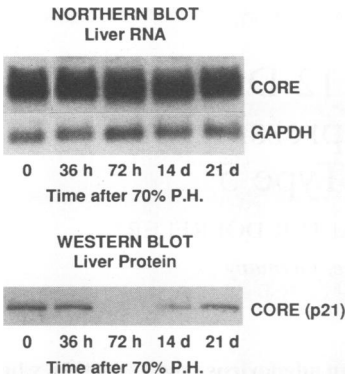

FIG. 7. Kinetics of loss and reappearance of nuclear HBc/eAg following 70% PH. (Top) Northern blot analysis of 20 μg of total RNA extracted from the livers represented in Fig. 6. The filters were hybridized with HBV- and GAPDH-specific probes. (Bottom) The same specimens analyzed by Western blot analysis for HBc/eAg content.

and therefore unable to add to the pool of pregenomes and viral mRNAs needed for viral replication. Additionally, even if they do contain competent viral genomes, the intranuclear nucleocapsid particles cannot form infectious virions unless they enter the cytoplasm, and this should occur only in dividing hepatocytes. If this scenario proves to be correct, perhaps the preformed nucleocapsids could serve as a reservoir for the rapid assembly of new virions in regenerating hepatocytes that are stimulated to divide as other infected hepatocytes are destroyed during viral hepatitis. Given the obscure role that these prominent intranuclear nucleocapsids play in the HBV life cycle, further examination of the physical, molecular, and functional properties of these particles in the HBV-infected liver is clearly warranted.

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