Central Role of a Serine Phosphorylation Site within Duck Hepatitis B Virus Core Protein for Capsid Trafficking and Genome Release*

Viral nucleocapsids compartmentalize and protect viral genomes during assembly while they mediate targeted genome release during viral infection. This dual role of the capsid in the viral life cycle must be tightly regulated to ensure efficient virus spread. Here, we analyzed the duck hepatitis B virus (DHBV) infection model to analyze the effects of capsid phosphorylation and hydrogen bond formation. The potential key phosphorylation site at serine 245 within the core protein, the building block of DHBV capsids, was substituted by alanine (S245A), aspartic acid (S245D) and asparagine (S245N), respectively. Mutant capsids were analyzed for replication competence, stability, nuclear transport, and infectivity. All mutants formed DHBV DNA-containing nucleocapsids. Wild-type and S245N but not S245A and S245D fully protected capsid-associated mature viral DNA from nuclease action. A negative ionic charge as contributed by phosphorylated serine or aspartic acid-supported nuclear localization of the viral capsid and generation of nuclear superhelical DNA. Finally, wild-type and S245D but not S245N virions were infectious in primary duck hepatocytes. These results suggest that hydrogen bonds formed by non-phosphorylated serine 245 stabilize the quaternary structure of DHBV nucleocapsids during viral assembly, while serine phosphorylation plays an important role in nuclear targeting and DNA release from capsids during viral infection.

Hepadnaviruses are a group of small enveloped DNA viruses with a narrow host range and a relative tropism for the liver. Hepatitis B virus (HBV),1 the prototypic member of the hepadnavirus family, is a major cause of liver disease worldwide, ranging from acute and chronic hepatitis to liver cirrhosis and hepatocellular carcinoma (1, 2). Other members of the family ranging from acute and chronic hepatitis to liver cirrhosis and hepatocellular carcinoma (1, 2). Other members of the family include the duck hepatitis B virus (DHBV) and the woodchuck hepatitis virus (WHV) (3, 4). Similar to other viruses the hepadnaviral genome is protected by a nucleocapsid, which is formed by icosahedrally arranged core proteins. The C-terminal domain of the core protein is rich in basic residues and bears three conserved serine phosphorylation sites (5, 6). While dispensable for capsid assembly, this region is required for packaging of pregenomic RNA (7–11). Inside the capsid the viral polymerase converts pregenomic RNA into minus-strand DNA, which in turn is completed to a double-stranded, relaxed circular (rc) DNA molecule (12). Newly assembled, mature hepadnaviral capsids containing rcDNA have two possible fates: they can either be enveloped by surface proteins and enter the secretory pathway or be redirected to the nucleus where repair of the rcDNA molecule results in the formation of covalently closed circular (ccc) DNA, the template for viral RNA synthesis. Nuclear translocation of viral rcDNA is also mediated by incoming capsids in newly infected cells. However, the precise mechanisms regulating capsid trafficking and uncaging in both settings are unknown.

Earlier studies in the DHBV model have shown that capsids from infected livers are highly phosphorylated while serum-derived capsids and particles secreted from cultured cells are dephosphorylated (13, 14). Furthermore, binding of in vitro generated HBV capsids to the nucleus of digitonin-permeabilized hepatoma cells was previously found to depend on the phosphorylation status of the core protein (15). Hepadnaviral capsid phosphorylation, therefore, represents a candidate mechanism for the regulated nuclear targeting and release of viral rcDNA during natural infection.

In the present report we used genetically engineered phosphorylation mutants of DHBV core protein to test this hypothesis. The duck model offers the advantage that primary duck hepatocytes (PDH) are readily available and that DHBV infection of PDH with supernatants from transfected cells is highly efficient and reproducible. Position 245 within the C terminus of DHBV core was selected for detailed analysis since this site of the core was previously found to influence capsid stability (17). In addition to the effect of ionic charge at this position we also analyzed the potential effect of hydrogen bond formation. Our data suggest that hydrogen bonding is required for the stabilization of newly assembled mature capsids while a negative ionic charge at position 245 of the DHBV core protein plays an essential role in nuclear capsid transport and infectivity. Based on these results we propose a model for multiple capsid functions based on the coordinated core protein phosphorylation and dephosphorylation, hydrogen bond formation as well as DNA maturation.

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1 The abbreviations used are: HBV, hepatitis B virus; DHBV, duck hepatitis B virus; MOPS, 4-morpholino-3-propane-sulfonic acid; PBS, phosphate-buffered saline; cccDNA, covalently closed circular DNA; TRITC, tetramethylrhodamine isothiocyanate; FITC, fluorescein isothiocyanate.

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**EXPERIMENTAL PROCEDURES**

**Cloning and Transformation**—Mutant S245A and mutant S245D were generated by cloning the 391-bp EcoRI/AvrII fragment of the corresponding expression vector described by Yu and Sedgwick (11, 16) into the overlength DHBV genome plasmid pOL16 (18). Mutant S245N was generated by cloning a PCR amplification product, in which the serine 245-coding triplet TCC was substituted by the asparagine-coding triplet AAC. The digested PCR fragment was ligated into EcoRl/BglII-digested overlength DHBV genome plasmid pOL16. The mutations were confirmed by DNA sequencing. To eliminate expression of the large surface protein, the 809 bp AvrII/Zohl fragment from plasmid 1165A (19) was ligated into the mutants described above. Chicken hepatoma LMH cells were seeded in 10-cm dishes and transfected by the calcium phosphate method (20). Cells were harvested on day 3 post-transfection as described below.

**Western Blot Analysis**—Transfected cells were detached from the culture dish by treatment with trypsin and resuspended in culture medium containing 5% fetal calf serum. The cells were sedimented and dissolved in 0.4 ml of Lämmli loading buffer containing 5% β-mercaptoethanol. To reduce viscosity, the lysate was passed through QIAshredder centrifugal devices (Qiagen) before loading on a 12% SDS-polyacrylamide gel. Proteins were transferred onto polyvinylidene difluoride membranes (Schleicher & Schuell) by electroblotting. Proteins were detected with a DHBV core-specific rabbit antiserum described by Schlicht et al. (21) and visualized by ECLplus chemiluminescence (Amersham Biosciences).

**Extraction and Detection of Viral DNA**—Transfected cells were harvested as described above. To disrupt plasma membranes, cells were resuspended in chilled 1 ml of isotonic buffer (140 mM NaCl, 1.5 mM MgCl_2, 50 mM Tris-HCl (pH 8.6) containing 0.5% Nonidet P-40. Nuclei were removed by centrifugation for 5 min at 14,000 × g. A 0.4-ml aliquot of cytoplasmic extract was digested with 300 units/ml microccocal nuclease (Amersham Biosciences) for 5 h at 37 °C in the presence of 2 mM CaCl_2. The precipitate formed during incubation was removed by centrifugation, followed by inactivation of the nuclease by EDTA (final concentration, 5 mM). Viral DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) including RNase A treatment as described above.

**Immunofluorescence Staining of Intracellular Core Protein**—HuH-7 cells were seeded on collagen-coated cover slips and transfected with the TfX-20 reagent (Promega). Starting on day 1 post-transfection, the cells were kept in starvation culture medium (Dulbecco’s modified Eagle’s medium containing 1% fetal calf serum) for 4 days. Afterward, the cells were fixed with 3% paraformaldehyde in PBS for 20 min at room temperature, followed by rinsing the cover slips with PBS. Cell membranes were permeabilized by 0.1% Triton X-100 in PBS for 10 min at room temperature, followed by two washes with PBS. Unspecific binding sites were blocked with blocking buffer (1% bovine serum albumin and 10% goat serum in PBS) at 37 °C for 30 min. For immunostaining, the cover slips were incubated for 90 min at 37 °C with blocking buffer containing 1:200 diluted rabbit anti-DHBV core serum (D69, kind gift from H. Schaller, ZMBH, Heidelberg, Germany). After four washes with PBS at room temperature, the secondary antibody (affinity-purified FITC-labeled goat anti-rabbit, H + L Dianova) was exposed to the cells for 40 min at 37 °C at a dilution of 1:200. All incubations at 37 °C were performed in a humidified box. After immunostaining, the cells were washed four times at room temperature with PBS, followed by 10 min of incubation with 10 μg/ml propidium iodide in PBS and four additional washes with PBS. Afterward, the cells were embedded in DABCO (Fluka Chemicals) containing 50 mg/ml methanol (Hoechst). Localization of core protein and cell nuclei was performed by confocal immunofluorescence microscopy (Leica DM IRBE microscope) using the TRITC- and FITC-fittings at a pinhole size of 1.

**Gradient Sedimentation of Cellular Nucleocapsids**—Cytoplasmic extracts were prepared as described above and sedimented through a 10–50% (w/v) Nycodenz (Nycomed Pharma AS) gradient. Nycodenz was dissolved in isotonic buffer (140 mM NaCl, 1.5 mM MgCl_2, 50 mM Tris-HCl, pH 8.0) containing 0.5% Nonidet P-40. Approximately 0.4 ml of cytoplasmic extract was loaded onto a 1-ml gradient in polycarbonate tubes. Samples were centrifuged at 55,000 rpm for 40 min at 20 °C in a TLS55 swing-out rotor (Beckman Instruments). Nine 0.15-ml fractions were collected from the top of the gradient (where cellular proteins floated on the top of the gradient, whereas viral nucleocapsids sediment into lower fractions). An aliquot of each fraction was mixed with Lämmli loading buffer containing 5% β-mercaptoethanol and analyzed by Western blotting. A second aliquot was processed for DNA extraction. Purified DNA was digested with PsI before agarose gel electrophoresis and Southern blotting.

**Gradient Centrifugation of Extracellular Particles**—Supernatant from LMH cells was collected on day 3 post-transfection and concentrated using the UltraFree Biomax 100K centrifugal filter devices (Millipore). From one cell culture dish, 5 ml of supernatant was concentrated to a final volume of 0.4 ml. Concentrated supernatant was loaded onto a 1-ml gradient containing 0.04 ml of 1:100 dilution of chloroform in 100 mM NaCl, 10 mM Tris-HCl, pH 7.5. Centrifugation was performed for 20 h at 55,000 rpm at 10 °C in a TLS55 swing-out rotor. Enveloped DHBV particles accumulate in the middle of the gradient at a density of 1.2 g/ml, while non-enveloped nucleocapsids sediment at a density of 1.3 g/ml (14, 22). The gradient fractions were harvested separately above and below the interface, treated with 10 μg/ml propidium iodide, followed by DNA extraction, PsI digestion, and Southern blotting.

**Infection of Primary Hepatocytes**—Primary hepatocytes were prepared from DHBV negative ducklings by two-step collagenase liver perfusion as described before (23). Hepatocytes were maintained in Williams E medium supplemented with 1 μM dexamethasone, 15 μM HEPEIS pH 7.3, 1 μM insulin and 1 μM transferrin. From transfected LMH cells were collected on day 3 post-transfection and concentrated using UltraFree Biomax 100K centrifugal devices (Millipore). Viral particles were added to primary hepatocytes on day 1 post-seeding. Supernatant from one 10-cm dish was transferred to three
wells of a 6-well plate with primary hepatocytes. 4-h postincubation, the culture medium was exchanged, followed by daily medium changes. To harvest the cells, culture medium was removed, and hepatocytes were dissolved in 0.4 ml of ATL lysis buffer. DNA was extracted by adsorption onto QIAamp columns and digested with PstI before agarose gel electrophoresis and Southern blotting.

RESULTS

Design and Replication Competence of DHBV Capsid Mutants—Three overlength constructs were generated in which serine 245 of the DHBV core protein was substituted by alanine (S245A), aspartic acid (S245D), or asparagine (S245N). Aspartic acid and alanine are generally accepted standard substitutions of serine to mimic the phosphorylated and non-phosphorylated state, respectively. Asparagine, which is structurally similar to aspartic acid, was included to also assess the potential influence of hydrogen bonds formed by non-phosphorylated serine. The 3’-region of the hepadnaviral core open reading frame, which encodes the C-terminal domain of core, overlaps with the 5’-region of the viral polymerase open reading frame. For this reason site-directed mutagenesis in mutants S245D and S245N resulted in a concomitant substitution of the polymerase proline 64 by threonine. In mutant S245A the polymerase protein was unchanged.

First, we investigated the expression levels of the respective core protein mutants by Western blot analysis after transfection of LMH cells. As shown in Fig. 1, all mutants produced wild-type levels of core protein.

Next we analyzed the replication competence of the respective mutants. As shown in Fig. 2A, left panel, all mutants were replication competent. The overall level of replication was reduced to different degrees, however. For mutants S245D and S245N this finding is most likely due to the altered polymerase sequence, as revealed by trans-complementation experiments with a core-deficient overlength construct (data not shown). In the case of S245A, however, a direct effect of the core protein must be assumed. Northern blot analysis of total and capsid-associated RNA showed that RNA encapsidation was not rate from rcDNA or whether rcDNA remains associated with viral plus strand DNA synthesis and become physically separate from rcDNA or whether rcDNA remains associated with core protein. To address this issue S245A capsids were further analyzed by immunoprecipitation and density gradient sedimentation. Fig. 3 shows that rcDNA specifically co-precipitated with wild-type as well as mutant core protein. When subjected to density gradient sedimentation, rcDNA co-localized with particulate core protein (Fig. 4). Furthermore, there was no

![Fig. 1. Expression levels of mutant DHBV core proteins. LMH cells were transfected with overlength DHBV genome constructs and analyzed by Western blotting. Core protein residue 245 was wild-type (S), aspartic acid (D), alanine (A) or asparagine (N). nt, non-transfected control; 33 kDa, protein size marker.](image1)

![Fig. 2. Viral DNA synthesis, packaging of pregenomic RNA and nuclease-sensitivity of capsid-associated DNA. A, viral DNA synthesis. LMH cells were transfected as indicated on top of the figure. Cytoplasmic extracts were either treated with micrococcal nuclease (+ MN, right panel) or left untreated (− MN, left panel) prior to DNA extraction and Southern blot analysis. For the nomenclature of the respective mutants see Fig. 1. B, transfection plasmid DNA; RC, relaxed circular DNA; SS, single-stranded DNA. As quantified by densitometry the overall replication level of the respective constructs was as follows. Left panel, S 100%, D 81%, A 49%, N 69%; right panel, S 94%, D 57%, A 25%, N 56%. B, packaging of pregenomic RNA. LMH cells were transfected as indicated on top of the figure. Cytoplasmic extracts were either treated with micrococcal nuclease (+ MN, right panel) or left untreated (− MN, middle panel) prior to the extraction of RNA and subsequent Northern blot analysis. pgRNA, pregenomic RNA. C, nuclease titration assay of S245A-associated rcDNA. Cytoplasmic extract from S245A-transfected cells (left panel) and cytoplasmic extract from non-transfected cells mixed with purified rcDNA (right panel) were treated with serially diluted micrococcal nuclease before DNA extraction and Southern blotting. For abbreviations, see legend to Fig. 2.](image2)
evidence for an accumulation of monomeric/dimeric core protein relative to particulate core protein, as would have been expected in the case of complete capsid disintegration. Identical results were obtained for mutants S245D and S245N (data not illustrated). It should be noted, however, that empty or RNA-containing capsids cannot clearly be distinguished from rcDNA-containing capsids in this assay. Plasmid DNA also entered the gradient in this experimental setting, most likely as calcium phosphate complexes. Importantly, however, the rcDNA peak co-localized with the core protein but not with plasmid DNA, indicating that the sedimentation pattern of rcDNA was not due to a nonspecific mechanism. This interpretation is confirmed by the fact that purified rcDNA did not enter the gradient (data not shown).

Mutants S245A and S245N Are Defective in Nuclear Targeting and cccDNA Formation—Next we investigated the potential effect of DHBV core protein phosphorylation on intracellular trafficking and cccDNA formation. Preventing viral surface protein synthesis enhances capsid recycling to the nucleus and cccDNA formation (19). We therefore introduced a stop codon within the surface open reading frame of the respective over-length constructs. The subcellular localization of core protein mutants was assessed by confocal immunofluorescence laser-scan microscopy of transfected HuH-7 cells. Fig. 5 shows a representative panel of core-positive cells for each mutant. While the staining pattern was not strictly homogenous, blinded analysis of each individual mutant revealed that wild-type and mutant S245D reproducibly generated intranuclear as well as cytosolic core protein. By contrast, the subcellular localization of core was predominantly cytoplasmic for mutants S245A and S245N with individual cells displaying a moderate nuclear core staining. This observation may be due to impaired nuclear import or accelerated nuclear export of core protein (24). Identical results were obtained in LMH cells (data not shown).

To assess cccDNA formation in the presence and absence of large surface protein expression we analyzed nuclei from transfected LMH cells by Southern blotting. As expected, only the surface-negative variants yielded strong cccDNA signals (Fig. 6A). For wild-type and mutant S245D high levels of cccDNA were observed. By contrast, cccDNA formation remained at lower levels for S245A and S245N (Fig. 6A, right panel and Fig. 6B). This finding was confirmed in three independent experiments.

All Mutants Produce Enveloped Particles—We then investigated the competence of mutant capsids to enter the secretory pathway. Culture media of transfected cells were centrifuged to equilibrium in a cesium chloride gradient and fractions were analyzed by Southern blotting. Fig. 7 shows that all mutants were enveloped. Compared with wild-type, however, the respective levels in virion production were reduced. In particular, enveloped S245A particles were only detectable after prolonged exposure of the Southern blot. This result is best explained by the reduced overall replication rate of mutant S245A and by its selective deficiency of mature, rcDNA containing capsids.

Mutant S245N Is Not Infectious in Primary Hepatocytes—Finally, the respective mutants were tested for their ability to infect primary duck hepatocytes. Due to the low amounts of enveloped virions obtained with mutant S245A, this study was restricted to mutants S245D and S245N. Supernatants of transfected LMH cells were added to primary duck hepatocytes, and the cells were harvested at the time points indicated. Successful infection was demonstrated by the accumulation of cccDNA and newly synthesized replicative intermediates. Productive infection of primary duck hepatocytes (PDH) by wild-type virions is shown in Fig. 8, A and B, left panels. Mutant S245D was also infectious, but the efficiency of infection was reduced with a faint signal of cccDNA becoming visible on day 7 postinfection (Fig. 8A, right panel). S245N virions attached efficiently to primary duck hepatocytes (Fig. 8B, right panel). Nevertheless, cccDNA and newly synthesized replicative intermediates were not detectable in cells inoculated with this mutant virus, though rcDNA originating from the inoculating virus remained visible up to 9 days in culture. Thus, mutant S245N generated stable capsids, which were unable to initiate a productive infection in PDH. In addition, this result rules out that the generation of replicative intermediates observed for wild-type and mutant S245D originated from transfection of residual plasmid DNA present in the inoculum rather than from a true infection.

DISCUSSION

Taken together our results indicate that serine at position 245 of the DHBV core protein plays a key role in regulating the structural integrity and intracellular trafficking of mature, rcDNA containing nucleocapsids during different stages of the viral life cycle, most likely via its phosphorylation status.

Wild-type and S245N capsids were nuclease-resistant while encapsidated mature viral DNA generated by mutants S245D

FIG. 3. Relaxed circular DNA coprecipitates with S245A mutant core protein. Cytoplasmic extracts from LMH cells transfected with wild-type (WT, left panel) or the S245A construct (right panel) were subjected to immunoprecipitation. DNA was extracted from the first supernatant (Su) or from the pelleted Sepharose beads (Pe) after extensive washing and visualized by Southern blotting. Anti-core, polyclonal DHBV core-specific rabbit antibodies; mock, serum from a non-vaccinated rabbit. For abbreviations, see legend to Fig. 2.

FIG. 4. Gradient sedimentation of viral nucleocapsids. Cytoplasmic extracts from transfected LMH cells were loaded onto a 10–50% Nycodenz gradient and centrifuged for 40 min at 200,000 × g. Nine fractions were collected from the top of the gradient, and analyzed for viral DNA by Southern blotting (upper panel) and for core protein by Western blotting (lower panel), respectively. A, wild-type control; B, mutant S245A. Fractions 7, 8, and 9 contain the bulk of viral DNA as well as the bulk of core protein. The faint signals in fractions 2 and 3 represent non-specifically detected cellular proteins that are also present in the non-transfected control (see Fig. 1, nt). Note that viral DNA co-sediments with particulate core protein. Mutant S245D and mutant S245N yielded exactly the same sedimentation profile as the wild-type control (data not shown). For abbreviations, see legend to Fig. 2.
and S245A was nuclease-sensitive. Possible reasons accounting for this observation include differences in local protein structure preferred by alanine and aspartic acid compared with serine and asparagine and/or electrostatic repulsion of DNA by aspartic acid. Furthermore, hydrogen bonding mediated by non-phosphorylated serine as well as asparagine may contribute to the stabilization of the quaternary structure of mature DHBV capsids. The precise structural properties of mutant capsids that are associated with the nuclease-sensitivity of mature viral DNA remain to be further elucidated. We obtained no evidence for a complete disintegration of mature S245A capsids in immunoprecipitation experiments and the density gradient sedimentation assay. It should be noted, however, that partially dissociated S245A capsids may have reassembled after DNA release with rcDNA remaining attached to the outside of the capsid. Alternatively, the sensitivity of rcDNA may simply be based on an increased “breathing” of the capsid. This phenomenon involves the slow release of capsid subunits followed by a rapid reformation of the capsid integrity, as discussed previously for HBV capsids (25).

The fact that only mature rcDNA but not ssDNA associated with mutant capsids S245D and S245A was accessible to nuclease action indicates that DNA maturation is an important trigger of capsid destabilization. Possibly, interactions of viral DNA with the C terminus of the core protein are of importance in this context (26). Our results shed new light on previous data, which had implicated that the C-terminal domain of DHBV core protein is required for DNA maturation. Rather, this report and our previous study on deletion and insertion mutants of the DHBV core protein (17) suggest that the phenotype of several mutants is at least in part due to nuclease sensitivity of mature viral DNA. The fact that nuclease treatment is commonly used to remove plasmid DNA prior to extraction of nucleocapsid associated hepadnaviral DNA explains why fully mature DNA forms have not been observed earlier. Indeed, we found that nuclease sensitivity of rcDNA also accounts for the phenotype of a previously described P246G mutant of DHBV core (16). It will be interesting to determine the relevance of these observations for HBV core protein mutants.

Our data suggest that negative charges at position 245 as contributed by phosphorylated serine or aspartic acid support nuclear targeting of DHBV core protein, cccDNA formation and infectivity. The residual cccDNA formation by mutant S245N could be due to nuclear import of small amounts of core protein mediated by the remaining, unchanged phosphorylation sites. This may be difficult to detect by immunofluorescence since the polyclonal anti-DHBV core antibody may not react well with core protein dimers in fixed cells. Alternatively, capsids might become entrapped non-specifically within the nucleus of dividing cells and be degraded subsequently. Nevertheless, our infection experiments in PDH demonstrate that the residual cccDNA formation by S245N does not suffice to initiate productive infection.

Collectively, our observations suggest the following model: maturation of rcDNA within the nucleocapsid is an important trigger of capsid destabilization, which ultimately results in...
DNA release. During viral assembly hydrogen bonds formed by non-phosphorylated serine 245 contribute to the stabilization of nucleocapsids thus preventing DNA liberation in the cytoplasm. During infection, phosphorylation of serine 245 directs the capsid to the nucleus. At the same time nucleocapsids are destabilized thus facilitating rcDNA release in the nucleus.

These mechanisms would also apply to progeny capsids that are redirected to the nucleus for cccDNA amplification. How newly formed DHBV capsids are directed to the secretory pathway remains to be elucidated, however, since Mabit and Schaller (13) have shown that dephosphorylation alone is not sufficient to confer membrane affinity. The suggested model is in line with the previous observation in the duck model that serum-derived nucleocapsids are non-phosphorylated whereas nucleocapsids from liver tissue are highly phosphorylated (14). It is tempting to speculate that incoming and recycling capsids are phosphorylated by a capsid-associated kinase activity of cellular origin, which has been described in the WHV and HBV system (27–33).

The present report demonstrates a central role of serine 245 in regulating DHBV capsid trafficking and stability. Clearly, however, the remaining phosphorylation sites within DHBV core may play an important role as well, since earlier reports indicate that serine 259 also affects viral infectivity (10, 16). It remains to be elucidated which and how many residues of individual nucleocapsids are phosphorylated at a given time point and how core protein phosphorylation is orchestrated during capsid assembly and disassembly. Further studies in the HBV and WHV system will have to establish whether mammalian core protein phosphorylation is essential for infectivity as well. The recently described infection of primary tu-pain hepatocytes (34) might represent a suitable model to address this question. Interestingly, phosphorylation of capsid protein by capsid-associated kinase activities has also been suggested to play a key role during the early steps of HIV infection (35). Thus, phosphorylation might represent a general mechanism regulating the targeted genome release from viral capsids.

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