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Morphogenesis of hepatitis B virus and its subviral envelope particles

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Summary

After cell hijacking and intracellular amplification, nonlytic enveloped viruses are usually released from the infected cell by budding across internal membranes or through the plasma membrane. The enveloped human hepatitis B virus (HBV) is an example of virus using an intracellular compartment to form new virions. Four decades after its discovery, HBV is still the primary cause of death by cancer due to a viral infection worldwide. Despite numerous studies on HBV genome replication little is known about its morphogenesis process. In addition to viral neogenesis, the HBV envelope proteins have the capability without any other viral component to form empty subviral envelope particles (SVP) which are secreted into the blood of infected patients. A better knowledge of this process may be critical for future antiviral strategies. Previous studies have speculated that the morphogenesis of HBV and its SVP occur through the same mechanisms. However recent data clearly suggest that two different processes, including constitutive Golgi pathway or cellular machinery that generates internal vesicles of multivesicular bodies (MVB), independently form these two viral entities.

Introduction

The human hepatitis B virus (HBV) is a small hepatotropic and highly infectious DNA virus member of the *Hepadnaviridae* family. HBV represents the prototype virus of the *Orthohepadnavirus* genus. Discovered almost forty years ago (Dane *et al.*, 1970; Blumberg *et al.*, 1965), HBV still remains a major health problem worldwide, as there is no widely available treatment for the estimated 350 million chronic carriers who have a high risk of liver cirrhosis or cancer. Despite the existence of an effective vaccine designed more than twenty years ago, currently almost 1 million people per year die due to HBV infection (Perz *et al.*, 2006). A thorough understanding of HBV structure and morphogenesis is thus promptly required for the development of innovative antiviral treatment.

The infectious HBV virion (or Dane particle) is a spherical particle, 42 nm in diameter, consisting of an icosahedral capsid about 30 nm in diameter. The virion contains the circular partially double-strand genomic DNA about 3.2 kb in length which is covalently linked to the viral reverse-transcriptase. This nucleocapsid is surrounded by a lipid bilayer in which the three envelope proteins (small [S], medium [M] and large [L]) are anchored as transmembrane proteins playing a major role in HBV morphogenesis and infectivity (Bruss, 2007). After being delivered into the nucleus (Rabe *et al.*, 2003), the viral DNA is converted to a covalently closed circular DNA (cccDNA) which is transcribed in : (i) pregenomic RNA (pgRNA) about 3.5 kb in length and (ii) different subgenomic RNAs encoding all the viral proteins necessary for the HBV replication cycle (Beck and Nassal, 2007). In the cytosol, a single molecule of pgRNA together with the viral reverse transcriptase is incorporated into an assembling capsid. Once the pgRNA is encapsidated, reverse-transcription into a new genomic DNA occurs (Beck and Nassal, 2007). The mature nucleocapsid is then enveloped by the surface proteins and a new generation of infectious virions can be released (Bruss, 2007). Interestingly, the production of the HBV surface proteins also leads to the intracellular budding of empty subviral spherical or filamentous envelope particles (SVP). SVP are 20 nm in diameter, lack the nucleocapsid and are secreted in great excess over virions (Ganem, 1991; Heermann *et al.*, 1984). It has been clearly shown that this natural phenomenon specific to HBV is linked to the ability of the S envelope protein to spontaneously form empty envelope particles at the rough endoplasmic reticulum (ER) (Dubois *et al.*, 1980). This noninfectious material is currently the basis of most vaccines against hepatitis B (Eddleston, 1990). Unfortunately, despite *in vitro* production of virions using HBV DNA transfection of Huh-7 or HepG2 cells (Yaginuma *et al.*, 1987; Sureau *et al.*, 1986) or HBV infection of the recently developed HepaRG cell line (Gripon *et al.*, 2002), HBV morphogenesis remains highly difficult to observe by electron microscopy (EM). This may be due to the low rate of HBV production approaching 1 to 10 viruses per hepatocyte per day *in vivo* (Nowak *et al.*, 1996). Thus, the models of HBV and its subviral envelope particles morphogenesis are based on biochemical approaches (Huovila *et al.*, 1992) and lack ultrastructural data that may be helpful for a more complete understanding of these mechanisms, as for other viral models (Roingeard, 2008). In this review, we focus on data that have recently been accumulated on both

HBV and SVP morphogenesis and illustrate our discussion with new and original EM observations.

HBV capsid

The 22 kD HBV core protein (also called C or HBc) consists of 183 or 185 amino acid (aa) residues depending on the genotype and is relatively well-conserved among HBV isolates (Chain and Myers, 2005). In the cytosol, the fundamental building unit of the HBV capsid is formed by homodimerization of two core proteins stabilized by a disulfide bridge between their respective Cys-61 residues (Zheng *et al.*, 1992; Zhou and Standring, 1992). Then, dimer oligomerization occurs by unclear cellular processes and leads to the formation of two different types of capsids : (i) a capsid consisting of 90 dimers associated in an icosahedral symmetry (T = 3) about 30 nm in diameter and (ii) a larger capsid consisting of 120 dimers associated in an icosahedral symmetry (T = 4) about 34 nm in diameter (Wynne *et al.*, 1999). Both capsids are found in infected cells but the T = 4 capsids seem to be preferentially selected for envelopment in infectious particles (Roseman *et al.*, 2005). However T = 3 capsids have also been reported to be incorporated in virions in various percentages (Dryden *et al.*, 2006; Roseman *et al.*, 2005). While the N-terminus of the capsid protein is important for the dimerization process, the basic C-terminal domain contains a nuclear localization sequence (NLS) and an Arg-enriched area. Formed by the last 34 aa, this basic area is probably involved in the pgRNA / reverse-transcriptase complex encapsidation (Nassal, 1992; Gallina *et al.*, 1989). Finally, the HBV core protein structure was solved by crystallization at a resolution of 3.3 Å (Wynne *et al.*, 1999). In a recent review, Bruss described the HBV core dimer as a structure resembling an upside down “T” ; with the horizontal bar mediating the inter-dimer association (Bruss, 2007). The perpendicular domain to this bar consists of a spike formed by 4 α-helices, 1 α-helical hairpin for each protein, protruding outwards from the center of the dimer basis (Wynne *et al.*, 1999). The sequence from residue 78 to residue 83, located on the top of the spike, is known as the major epitope of the capsid antigen (HBcAg). Associations of 5 or 6 dimers are arranged around the 12 axes with a five-fold or two-fold (or quasi six-fold) symmetry, respectively. Finally, analysis by cryo-electron microscopy (cryo-EM) showed that the capsid was not continuous but opened by the presence of pores about 12 to 15 Å in diameter (Wynne *et al.*, 1999). It was suggested that these pores are essential to enable the entry of deoxyribonucleotides during the genomic DNA synthesis and / or the expulsion of ribonucleotides after the degradation of the pgRNA by the RNaseH activity of the reverse transcriptase.

As described above, all stages of pgRNA reverse-transcription occur in the nucleocapsid. However, only virions containing a mature circular partially double-strand genomic DNA are secreted into the extracellular environment (Weiser *et al.*, 1983; Mason *et al.*, 1982). Actually, it has been suggested that the presence of the pgRNA could interfere with the incorporation of the nucleocapsid in the virion. The synthesis of genomic DNA would be required and associated with structural changes in the capsid structure leading to a mature nucleocapsid formation capable of being wrapped by the viral envelope (Summers and Mason, 1982). Recently, Roseman *et al* were able to support this hypothesis by demonstrating a structural difference between a pgRNA-containing capsid and a genomic DNA-containing capsid. The difference is related to the contact force generated by a double-strand nucleic acid on

a hydrophobic pocket located at the junction between the spike and the basis of the dimer (Roseman *et al.*, 2005). This pressure would make the pocket accessible to the HBV surface proteins in order to proceed to the nucleocapsid / envelope interaction. Interestingly, the natural mutation I97L found in the "*adr*" HBV subtype leads to the early envelopment of an immature single-strand DNA-containing nucleocapsid which can be corrected by the P130T point mutation (Yuan and Shih, 2000). In addition, the high infectivity of HBV could be explained by the existence of a quasi-control and/or a strict selection for mature nucleocapsid envelopment and new infectious virions secretion. As a typical example, the duck hepatitis B virus (DHBV, genus *Avihepadnavirus*) production rate is about 1 infectious virion per physical particle secreted (Jilbert *et al.*, 1996).

HBV envelope

The three envelope proteins S, M and L are encoded by a single open reading frame referred as ORF-E, 389 or 400 codons in length depending on the "*ay*" or "*ad*" viral subtype, respectively. This ORF consists of three 5' in-phase ATG codons for the initiation of translation and one 3' TAA termination codon. Thus, the three HBV envelope proteins only differ by the length of their N-terminal domains. These envelope proteins are present in different proportions in the three types of HBV-related particles : while the S protein represents the major component of the viral envelope, the L protein is only found in the virion and the filamentous SVP (Heermann *et al.*, 1984). The M distribution seems to be the same in all particles.

The 226 aa S protein is a typical membrane protein with a relatively complex topology and is produced at the rough endoplasmic reticulum (ER) (Bruss, 2007). Computer modeling of its secondary structure suggests that three hydrophobic integral domains are separated by two hydrophilic loops. The S protein is translocated at the ER compartment by its N-terminal first transmembrane domain (TM-I) located between residues 4 to 24. The fact that this sequence is not cleaved by any of the cellular signal peptidases allows its co-translational translocation through the ER membrane in order to direct the N-terminus of the protein into the ER lumen. Downstream of this TM-I domain is a cytosolic loop (CYL-1) followed by a second transmembrane domain (TM-II) located between residues 80 to 100 which acts as a type II signal/anchor domain. The TM-II C-terminal end directs a luminal loop known as the antigenic loop (AGL) containing a single N-glycosylation site at Asn-146 (Peterson *et al.*, 1982). The prediction models for the third C-terminal hydrophobic domain show that this region is actually composed of two transmembrane α-helices TM-III and IV, composed of residues 173 to 193 and 202 to 222 respectively that are separated by a short cytosolic sequence (CYL-2) (Persson and Argos, 1994). The arrangement of these two helices in the ER membrane is probably responsible for the translocation of the C-terminus of S into the ER lumen (Sonveaux *et al.*, 1994). Since S contains fourteen Cys residues some of which are directly involved in disulfide bridge formation and/or particle secretion, it is clear that the acquisition of a functional structure must be extremely regulated (Mangold and Streeck, 1993). Therefore, as with many other transmembrane proteins, the co and post-translational modifications of S are highly monitored by ER chaperones such as the protein disulfide isomerase (PDI) (Helenius and Aebi, 2001; Huovila *et al.*, 1992). Several other chaperones are likely involved in this control but still remain undefined. The fact that only half of the small envelope proteins are found N-glycosylated in the

particles clearly emphasizes the complexity of its maturation (Peterson *et al.*, 1982). The S protein is an unusual viral surface protein in the sense that it is the necessary and sufficient unit for the formation and the spontaneous budding of HBV subviral envelope particles (SVP), in the absence of any other viral factor. Indeed, *in vitro* production of S alone results in the secretion of empty and non-infectious spherical SVP which are currently industrially produced to generate highly efficient and safe vaccines against HBV (Eddleston, 1990; Dubois *et al.*, 1980). Additionally, it is widely accepted that the oligomerization of several S proteins is required to initiate the SVP morphogenesis (Huovila *et al.*, 1992). The exclusion of any other cellular proteins and even envelope proteins from avian hepadnaviruses during this process suggests a specific control of the SVP formation and composition (Gerhardt and Bruss, 1995; Stibbe and Gerlich, 1982).

The biosynthesis of the 281 aa M protein is quite similar to S. This protein differs from S by the N-terminal addition of a 55 residue sequence named “preS2” which is co-translationally translocated into the ER lumen by the TM-I signal sequence (Eble *et al.*, 1990). In addition to the Asn-201 (Asn-146 on the S protein), the preS2 domain contains an N-glycosylation site at Asn-4 together with an optional O-glycosylation site at Thr-37 depending on the genotype (Tolle *et al.*, 1998; Heermann *et al.*, 1984). Interestingly, a strict correlation between glycan-dependent chaperone binding and secretion of M indicates that ER proteins such as calnexin promote folding and trafficking of M allowing particle secretion (Werr and Prange, 1998). However, the exact role of M in the viral cycle remains unclear. Indeed, its *in vivo* absence in infected cells does not disrupt viral morphogenesis or particle functionality (Fernholz *et al.*, 1991). Thereby, this protein seems not to be essential for virus spread and its complete absence from the *Avihepadnavirus* genomes reinforces this hypothesis.

The L protein is characterized by the addition of a 108 to 119 residue sequence named “preS1” on the N-terminus of M. Thus, this large protein basically incorporates the preS domain (preS1 and preS2) to the N-terminus of S. Although this has not been demonstrated experimentally, the N-terminus of preS1 is probably anchored on the ER surface by a fourteen carbon saturated fatty acid added by myristylation at Gly-2 (Persing *et al.*, 1987). The kinetics of this biochemical modification and its exact role for the virus life cycle are still unclear but cellular enzymes such as the cytosolic *N-myristyl transferase* are probably involved in this process. Contrary to the M protein, the L protein is only N-glycosylated at Asn located in the S domain (Bruss, 2007). Two other potential sites of N-glycosylation are also present at Asn-15 of the preS1 domain and Asn-4 of the preS2 domain but are not glycosylated because of their cytosolic localization (Heermann *et al.*, 1984). Indeed, it seems that initially the N-terminus of L is retained in the cytosol as an internal preS domain in a topology called “i-preS” (Bruss, 2007). The translocation of the preS domain into the ER lumen occurs in an obscure but probably post-translational mechanism leading to an external topology of L called “e-preS”. This dual orientation of L is physically linked to the ER membrane, suggesting the involvement of host-cell transmembrane transport machinery. However, an interesting computer-aided analysis suggests that oligomerized S domains could form a channel in the ER membrane for the preS transport (Berting *et al.*, 1995). In parallel, some studies led to the identification of a topogenic element mapped to a preS1 C-terminal sequence located between residues 70 and 94, termed cytosolic anchorage determinant (CAD). CAD interacts

with the cognate heat-shock protein Hsc-70 preventing preS co-translational translocation into an “e-preS” topology and glycosylation of the Asn-15 and Asn-4 of the preS1 and preS2 domains, respectively (Lambert and Prange, 2003; Loffler-Mary *et al.*, 1997). Luminal chaperones binding to the e-preS domain such as the binding protein BiP could support this process (Cho *et al.*, 2003). Currently, it is clearly recognized that both “i-preS” and “e-preS” topologies of L play a specific role in the viral cycle : recruitment of a mature viral nucleocapsid for virion budding (Bruss, 2007) and recognition of the cellular (co-)receptor(s) for virus entry (Glebe and Urban, 2007), respectively.

SVP morphogenesis

As described above, the HBV surface proteins are not only incorporated into the virion envelope but also bud in an empty and non-infectious subviral envelope particle (SVP). The SVP conformation is organized as an octahedral sphere about 20 nm in diameter containing 48 S-dimer subunits or as a filament with the same diameter but with variable length (Gilbert *et al.*, 2005). This variation in the particle topology seems to greatly depend on the L protein quantity and on the S/L ratio incorporated into the particle during the morphogenesis process. Thus, since the spherical SVP are essentially composed by the S protein, the filament organization is greatly dependent on the co-assembly of a large proportion of L protein together with S (Heermann *et al.*, 1984). During the infection, it is currently assumed that these SVP act as decoys, trapping the host’s immune system to protect the infectious virus. However, a high proportion of L protein tends to retain the particles inside the infected cell because of specific retention motifs (Persing *et al.*, 1986). Therefore, the inability of infected cells to export long HBV filamentous particles through the cellular secretion pathway seems to be at the origin of a direct cytopathic effect described as an inclusive cellular vesicle called “ground-glass” structure (Roingeard and Sureau, 1998).

How the SVP formation occurs in the cell is still highly debated, but recent ultrastructural observations of cells producing the small S protein alone have provided a better understanding of this particular question (Patient *et al.*, 2007). The structural unit of the SVP is a block composed by a strongly associated dimer of S protein (Gilbert *et al.*, 2005), and this dimer is probably stabilized by disulfide bridges which are generated under the control of the ER chaperone PDI (Huovila *et al.*, 1992). The S protein self-assembles initially in a filamentous form within the perinuclear space which is part of the ER of the cell (Fig 1A-a). These filaments are then clustered and packed into crystal-like structures and transferred by ER-derived vesicles to the rough ER-Golgi intermediate compartment (ERGIC) (Fig 1A-b). Unfortunately, the mechanisms and the cellular factors favoring all these events are not yet understood. In higher eukaryotes, transport from the ER is initiated by a COP-II-mediated budding of vesicular carriers through a specialized subdomain of the ER named ER-exit sites (ERES) (Appenzeller-Herzog and Hauri, 2006). Theorically the directionality of vesicle transit from one cellular compartment to another is dependent on a motor/cytoskeletal network including microtubule-plus-end-directed kinesin and/or microtubule-minus-end-directed dynein. However, as ERGIC clusters are close to but clearly distinct from ERES, ER to ERGIC transports are known to be microtubule-independent. The ER-derived vesicles containing the S filaments are then transported to the ERGIC where the membrane docking is probably mediated by

tethering proteins such as p115, a Rab1 effector, and followed by a SNARE-mediated membrane fusion. Once unleashed into the ERGIC lumen, the filaments are unpacked and relaxed possibly due to a different cellular milieu and/or association with new chaperones (Fig 1A-c), before being converted into spherical particles for secretion (Fig 1A-d). This hypothesis is supported by the observation that S secreted particles produced in a transgenic mouse model show a significant heterogeneity in size, a phenomenon that may be linked to the dissociation of filamentous particles (Gilbert *et al.*, 2005). Despite the lack of information indicating a specific release pathway of spherical SVP from the ERGIC, the conversion of the N-linked glycans on the S protein into complex structures before exocytosis provides arguments for the export of the particles using a constitutive cellular secretory pathway through the Golgi appartus (Patzer *et al.*, 1986). In all cases, COP-I and the Arf family of small GTPases may be involved.

While our understanding of the SVP formation and traffic has progressed, the early mechanisms of their assembly remain unclear. To illustrate this review, we have re-examined cells producing the HBV S protein by EM, paying particular attention to the perinuclear space, to identify the events that precede the budding of vesicles containing packed filaments (Fig 1B). This observation suggests that the filaments increase progressively in size and number and initially induce a slight dilatation of the perinuclear space (Fig 1B-a to 1B-d) before being clustered and packaged to initiate the budding of the subsequent transport vesicle. To date, no direct extrusion of the filaments from the membrane of the nuclear envelope has been observed, and intraluminal free ends were clearly identified for some filaments (arrow in Fig 1B-d). This suggests that these particles self-assemble inside the perinuclear space by a process quite distinct from lipid bilayer vesicle budding. This is supported by biochemical analyses that have shown that lipids which are only 25% by weight in the SVP have a highly restricted composition and are not organized in a typical bilayer configuration, but are instead located on the surface of the particle (Satoh *et al.*, 2000; Gavilanes *et al.*, 1982). The absence of lipid bilayer in the S SVP was also recently confirmed by cryo-EM analysis (Gilbert *et al.*, 2005).

HBV morphogenesis

Unlike some *Lentivirinae* able to bud through a lipid bilayer without any envelope protein (Hourioux *et al.*, 2000), the HBV nucleocapsid budding is strictly dependent on the large L protein. The absence of L or its *in vitro* production under an exclusive “e-preS” topology completely abolish the generation of new virions (Bruss and Vieluf, 1995). Indeed, the “i-preS” topology of L is obligatory for mature HBV nucleocapsid recruitment. A sequence overlapping the 17 C-terminal residues of the preS1 domain and the 5 N-terminal residues of the preS2 domain seems to be involved in this process (Bruss, 2007; Poisson *et al.*, 1997). Moreover, in order to take advantage of all the conditions in the cell, when the ratio between the “i-preS” L protein and the nucleocapsid is not optimal, the genomic viral DNA is preferentially recycled to the nucleus to amplify the cccDNA stock (Summers *et al.*, 1990). When an HBV virion is finally assembled, cryo-EM analyses show that the spike of the core dimer does not actually penetrate the lipid envelope but stays in close apposition by unclear biophysical links (Seitz *et al.*, 2007; Dryden *et al.*, 2006). A second point of interaction between 11 aa residues located at the basis of the core dimer and the preS domain of L also plays a role in particle stability (Ponsel and Bruss, 2003).

It has been generally presumed that the budding of SVP reflect the budding of HBV virions. However, although HBV is an enveloped DNA virus, there is growing evidence that HBV maturation and egress depend, as for many enveloped RNA viruses including *Retroviridae* or *Filoviridae*, on intraluminal vesicles of maturing endosomes known as multivesicular bodies (MVB) (Fig 2) (Lambert *et al.*, 2007; Watanabe *et al.*, 2007; Kian Chua *et al.*, 2006). Usually, MVB are involved in the sorting of misfolded and ubiquitinated proteins for their degradation (Slagsvold *et al*., 2006 for review). The proteins driving this machinery are grouped into at least three oligomeric endosomal sorting complexes required for transport ESCRT-I, -II and -III. These complexes are recruited and sequentially assembled to the cytosolic side of a late endosome membrane to generate MVB by multiple invaginations. Once the protein sorting is terminated, the disassembly of these multimers requires an ATPase associated with diverse cellular activities (AAA-type ATPase) named Vps4 (isoforms A or B) in humans. It has been previously shown that the ubiquitin-interacting adaptor γ2-adaptin and the Nedd4 ubiquitin ligase interact with the HBV core and L envelope proteins, possibly to regulate transport of the viral structures through the budding site (Rost *et al.*, 2006; Hartmann-Stuhler and Prange, 2001). Recently, several studies have demonstrated that the HBV core and envelope proteins co-localize with Vps4A/B and Alix/AIP1 (a functional tie between ESCRT-I and ESCRT-III in mammalian cells) and that dominant negative mutants of these host proteins inhibit virus release (Lambert *et al.*, 2007; Watanabe *et al.*, 2007). Thus, HBV virions would bud into late endosomes or MVB by utilizing ESCRT / Vps4B functions and exit the cell by the exosome pathway, as is the case for human immunodeficiency virus type-1 (HIV-1) in macrophages and human T-lymphoblastic cell lines (Grigorov *et al.*, 2006). This hypothesis is also supported by the presence of a PPAY sequence (residues 129 to 132) in the HBV core protein related to the tetrapeptide late-budding (L) domain PPxY (also PTAP or YPDL) found in retrovirus core proteins and involved in viral release through the MVB pathway (Slagsvold *et al.*, 2006; Wynne *et al.*, 1999). How ESCRT mediates viral budding is still unclear.

HBV and SVP assemble in different compartments

Recent studies on HBV morphogenesis have pointed out that the mechanisms of the SVP assembly are clearly distinct from those of the enveloped HBV virions and independent of the MVB functions. The γ2-adaptin, which strongly interacts with the HBV L protein does not recognize the HBV small (S) protein, the main component of the SVP (Rost *et al.*, 2006). Dominant negative mutants of Alix/AIP1 and/or Vps4A/B have no effect on the release of SVP (Lambert *et al.*, 2007; Watanabe *et al.*, 2007). Thus, the HBV virion and SVP assembly pathways differ in their requirement for cell functions and trafficking routes. Past EM studies of HBV-infected cells have documented that the virus assembles in large intracellular compartments (Roingeard, 2003; Roingeard and Sureau, 1998). Regarding the recent advances in understanding HBV morphogenesis, it appears that these large compartments were probably late endosomes or MVB. However, long subviral filaments appeared also to be formed by a tubular budding at the membrane of the same compartments (Roingeard, 2003; Roingeard and Sureau, 1998). Actually, it should be noted that these filaments were enriched in L protein. As the L protein interacts with the γ2-adaptin, it is probable that L-rich subviral filaments may assemble into MVB-related compartments by a conventional membrane-derived budding and by mechanisms quite distinct from those involved in the ER lumen for the HBV S filaments.

Conclusion

Contrary to the preexisting concept, HBV virion and subviral particle morphogenesis and traffic seem to involve specific cellular partners and specific secretion pathways. EM data obtained on cells producing the HBV S protein alone support the hypothesis that MVB functions required for HBV budding are not involved in the release of the SVP. The fact that these different particles accomplish their assembly in seemingly disparate ways is intriguing. The identity, recruitment, and mechanism of action of host factors involved in the various means of HBV and SVP assembly will be a challenge for future works. Technological advancements in mass spectrometry and large-scale proteome mapping will help to identify new interactions between viral and cellular proteins. Visualization of viral particles budding using real time high-resolution single-particle light microscopy and by using cryoelectron tomography and correlative electron microscopy techniques (Roingeard, 2008) may also shed light on these processes. As current therapy against HBV involves drugs that induce numerous unwanted side effects and may ultimately be rendered ineffective by drug resistant mutants, a better knowledge of these mechanisms might be a key factor in the development of more effective and better tolerated new anti-viral treatments.

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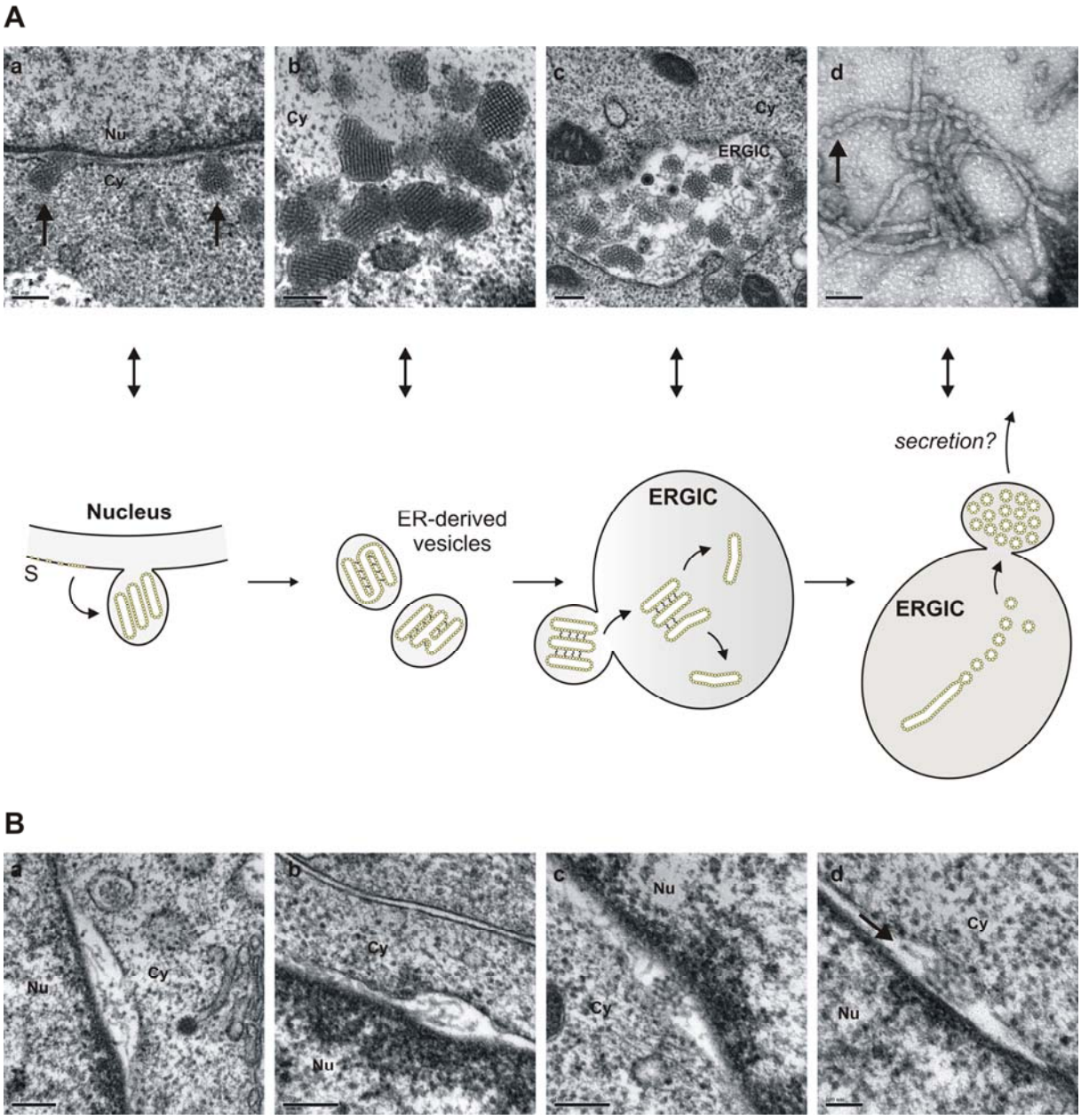


FIG. 1. EM observation of BHK-21 cells producing the HBV S protein (yellow dot, materials and methods described in Patient *et al*, 2007) and model for the SVP morphogenesis and traffic. Vesicles about 0.2 to 0.3 µm in diameter (arrows), packed with 20 nm-large HBV S filaments, are observed budding from the nuclear envelope (A-a). After budding, these vesicles are packed with HBV S filaments and appear in lengthwise or crosswise sections with a crystal-like structure (A-b). The fusion of such vesicles with the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) provides the relaxing of these filaments from their crystal-like structure in the ERGIC lumen (A-c). Negative staining of the purified intracellular HBV S filaments shows that these filaments tended to dissociate at their ends (arrow) into subviral spherical particles (A-d). A careful observation of the cell perinuclear space suggestes that nascent filaments are self-assembled in the lumen of this compartment rather than by membrane-derived budding (B-a to B-d). For some filaments, intraluminal free ends are identified (arrow in B-d). Bars in A-a, A-b, A-c, B-a, B-b and B-c, 200 nm ; Bars in A-d and B-d, 100 nm ; Nu, nucleus ; Cy, cytosol ; the cellular chaperones potentially involved in the SVP morphogenesis are presented as black zig-zag.

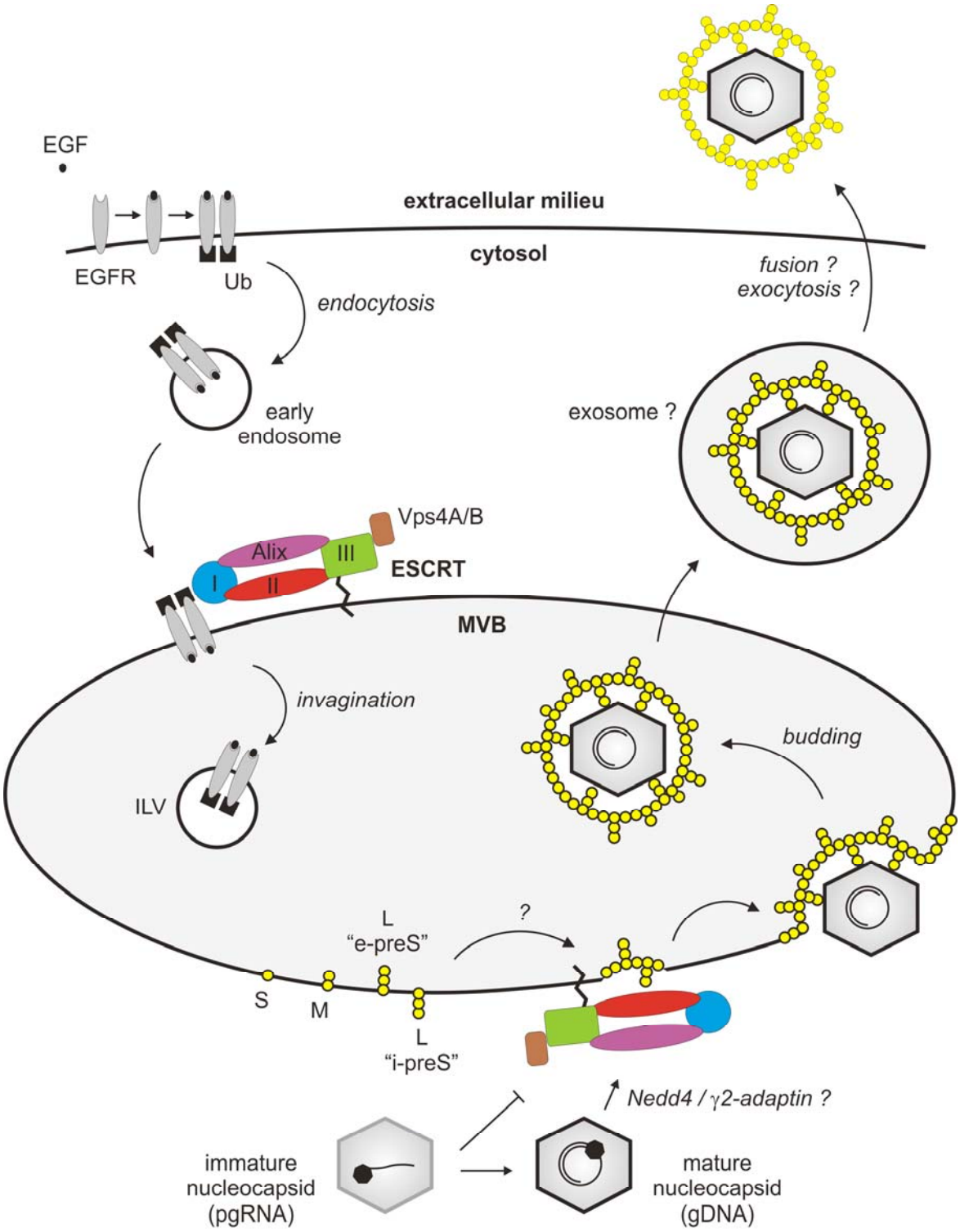


FIG. 2. Model for HBV morphogenesis. On the left is the representation of a typical endocytosis and multivesicular bodies (MVB) sorting of an activated membrane protein, *e.g.* the Epithelium Growth Factor Receptor (EGFR). The ubiquitylated (Ub, black square) protein is transported into an early endosome to the ESCRT machinery. In MVB, the ubiquitylated receptor is recognized by ESCRT-I (I, blue) which immediately recruits additional components of the MVB pathway : ESCRT-II (II, red), ESCRT-III (III, green) which is anchored to the MVB membrane by myristylation (zig-zag), Alix/AIP1 (Alix, purple) and Vps4A/B (brown) which is critical for disassembly of the complex following inward budding of intraluminal vesicle (IVL) into the MVB lumen. On the right, the hijacking of MVB sorting machinery for HBV morphogenesis is depicted. See text for details. pgRNA, pre-genomic RNA ; gDNA, genomic DNA ; the viral reverse-transcriptase located inside the capsid is presented as a black heptagon.