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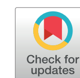
Review

Interconnection of cellular autophagy and endosomal vesicle trafficking and its role in hepatitis B virus replication and release



Jia Li [a], Yong Lin [b], Xueyu Wang [c], Mengji Lu [a,*]

[a] *Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, 45122, Germany*
[b] *Key Laboratory of Molecular Biology of Infectious Diseases (Chinese Ministry of Education), Chongqing Medical University, Chongqing, 400016, China*
[c] *The Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, 310000, China*

A R T I C L E I N F O

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A B S T R A C T

Hepatitis B virus (HBV) produces and releases various particle types, including complete virions, subviral particles with envelope proteins, and naked capsids. Recent studies demonstrate that HBV exploits distinct intracellular membrane trafficking pathways, including the endosomal vesicle trafficking and autophagy pathway, to assemble and release viral and subviral particles. Herein, we summarize the findings about the distinct roles of autophagy and endosomal membrane trafficking and the interaction of both pathways in HBV replication, assembly, and release.

1. Introduction

Hepatitis B virus (HBV) causes acute and chronic infection in humans. With an estimated 296 million chronically infected people worldwide, HBV infection remains a major public health problem (World Health Organization WHO, 2023). Chronic HBV infection is associated with an elevated risk of developing hepatitis, liver cirrhosis, and hepatocellular carcinoma (European Association for the Study of the Liver, 2017). Despite more than 30 years of intense research, many aspects of the HBV life cycle remain to be investigated to identify new targets for potential antiviral therapies (Revill et al., 2019). Research efforts are directed to understand viral pathogenesis and underlying molecular mechanisms.

HBV is one of the smallest enveloped DNA viruses with a 3.2-kb circular and partially double-stranded DNA genome, which is reverse transcribed from the pre-genomic RNA (pgRNA) and packaged in the capsid (Hu and Liu, 2017; Hu et al., 2019). The mature and infectious HBV virion is consisted of nucleocapsids (NCs) and surface proteins (HBsAg). There are three types of HBsAg (small, S; medium, M; and large, L). HBV produces empty particles consisting of HBsAg without capsid or genome, termed subviral particles (SVPs). SVPs are typically present in a 1000- to 100,000-fold excess relative to the infectious particles (Hu and Liu, 2017). SVPs can be observed in the forms of filaments and spheres. Notably, a considerable proportion of L- and S-HBsAg contributes to the formation of subviral filamentous particles, while spherical particles are predominantly composed of S-HBsAg (Patient et al., 2009). While there has been extensive investigation into HBV replication, assembly, and virion production within host cells, the specific manner in which autophagy and endosomal vesicle trafficking collaboratively facilitate HBV biogenesis remains an area that requires further exploration. Pertinent questions have emerged recently, encompassing HBV particle formation and degradation in association with autophagosome formation and maturation, the necessity of endosomal trafficking for the export of HBV virions and SVPs, as well as the redirection of HBV virions and SVPs from autophagosomes to the endosomal compartment and subsequent export pathway. This review aims to provide an in-depth overview of distinct roles of autophagy and endosomal vesicle trafficking and their connections in HBV replication, assembly, and release.

2. HBV assembly and secretion

The HBV infection cycle in hepatocytes includes receptor binding, entry, transport of capsids to the nuclear pore complex, covalently closed circular DNA (cccDNA) formation, transcription and translation, and assembly of capsids, viral particles, and SVPs, which have been comprehensively reviewed (Glebe and Urban, 2007; Yang et al., 2019). Cellular vesicle trafficking plays a crucial role in the HBV life cycle during viral entry, assembly, and release. HBV enters host cells through clathrin-mediated endocytosis by interacting with the Na^+-taurocholate co-transporting polypeptide (NTCP) (Herrscher et al., 2020; Yan et al., 2012). The process of HBV envelopment strictly depends on the presence

* Corresponding author.
E-mail address: mengji.lu@uni-due.de (M. Lu).

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of HBsAg. Early electron microscopic examinations suggested that HBsAg biosynthesis occurs as an integral transmembrane polypeptide, with assembly taking place in the endoplasmic reticulum (ER) lumen and subsequent transport to the pre-Golgi compartment (Huovila et al., 1992; Patient et al., 2007). S- and L- but not M-HBsAg are necessary for virion production (Bruss and Ganem, 1991). The pre-S domain of L-HBsAg is mainly responsible for the interaction with viral nucleocapsids (Bruss et al., 1994). The study conducted by Volker Bruss's group demonstrated that the pre-S sequence between Arg 103 and Ser 124 promotes HBV virion assembly (Bruss, 1997). HBcAg forms the icosahedral virus capsid and consists of 183 residues, which can be divided into an assembly domain and a domain responsible for viral genome packaging and replication (arginine-rich RNA-binding C Terminal Domain, CTD) (Liu et al., 2018; Prange, 2022; Venkatakrishnan and Zlotnick, 2016; Walker et al., 2011). Apart from the virologic factors, HBV assembly is also greatly influenced by cellular vesicle trafficking pathways, such as endosomal vesicle trafficking and autophagy, which will be elaborated in the subsequent discussion.

During the HBV replication cycle, hepatocytes independently secrete the infectious HBV virions (or Dane particles) and several species of incomplete viral particles, including SVPs/HBsAg, naked nucleocapsids, and empty virions, all of which can be detected in the blood of HBV infected patients (Hu and Liu, 2017). As much as 90% of secreted virion-like particles are found to be empty (Ning et al., 2011). These distinct HBV components utilize diverse pathways for their secretion. Spheres of a size of 20 nm, mainly consisting of S-HBsAg, are predominantly released via the constitutive ER-Golgi secretory pathway (Yang et al., 2021; Zeyen et al., 2020). Conversely, the release of L-HBsAg, responsible for the formation of filaments and virions, is mediated via endosomal sorting complexes required for transport (ESCRT)-dependent late endosomes/multivesicular bodies (MVBs) (Jiang et al., 2015; Ning et al., 2018; Zeyen et al., 2020). Filaments and capsids interact with ESCRT component TSG101 to facilitate their secretion (Jiang et al., 2015; Zheng Y. et al., 2023). Importantly, HBsAg that retained within the endoplasmic reticulum (ER) might trigger ER stress and contribute to viral pathogenesis such as the formation of ground glass hepatocytes (Li et al., 2011, 2019; Patient et al., 2007; Wang et al., 2003). It is proposed that the colocalization of HBc with Alix (apoptosis linked gene II interacting protein X) and HGS (hepatocyte growth factor-regulated tyrosine kinase substrate) facilitate the egress of naked capsids (Bardens et al., 2011; Chou et al., 2015). However, we should be very careful about the results since these host factors might also greatly affect cell biological activities like membrane transport thereby modulating virus replication and secretion. The formation of HBV virions has been found to be dependent on MVBs and necessitates the involvement of host factors γ2-adaptin, NEDD4, and ESCRT functions within the endosomal pathway (Lambert et al., 2007; Prange, 2012, 2022; Rost et al., 2006). The comprehensive exploration of how HBV exploits cellular biological activities to facilitate its assembly and secretion will be discussed thoroughly in the subsequent sections (Fig. 1).

3. Endosomal vesicle trafficking and its role in viral infection

Endosomes are dynamic and heterogeneous organelles that act as hubs for endocytic trafficking, recycling and degradation (Maxfield, 2014). The early endosomes serve as the starting point for late endosomes/MVBs maturation. Early endosomes receive endocytic cargos mainly through the clathrin-, caveolar-, GEEC-, and ARF6-dependent pathway (Mayor and Pagano, 2007; Mayor et al., 2014) Subsequently, the internalized cargos from early endosomes undergo distinct destines: recycling back to cell surface, maturing and assembling into late endosomes, or further being packaged into intraluminal vesicles (ILVs) within late endosomes/MVBs (O'Sullivan and Lindsay, 2020). Ultimately, MVBs fuse with cell plasma membrane, releasing their cargos into the extracellular space as exosomes, or delivering their contents to lysosomes for degradation (Huotari and Helenius, 2011). The transport of HBV from early to late endosomes/MVBs is essential in the viral life cycle, wherein Rab5 plays a key role in regulating the transport of endocytosed vesicles from the plasma membrane to early endosomes (Gorvel et al., 1991). Analyses of chronically HBV-infected liver tissue samples have demonstrated that endosomes serve as the main trafficking pathway for HBsAg, with a threefold higher colocalization of HBsAg with early endosomes marker Rab5a than with autophagosomes (Wang et al., 2022b). Furthermore, the uptake and transport of HBV core particles to early endosomes are dependent on clathrin-mediated endocytosis and Rab5, respectively (Cooper and Shaul, 2006). Another isoform of Rab5, Rab5b, has been observed to cause L-HBsAg accumulating in the ER upon its depletion (Inoue et al., 2019). The authors proposed that the depletion of Rab5b increased HNF4A transcription and led to the enhancement of L-HBsAg expression, and the retention of L-HBsAg was beneficial for HBV envelopment in the ER. Nonetheless, this hypothesis awaits further validation from subsequent studies. In an *in vitro* infection system, silencing Rab5 and Rab7 strongly reduced the production of encapsidated viral DNA (Macovei et al., 2013), suggesting that HBV transport through early endosomes is required for viral infection.

Previous studies indicated that matured late endosomes/MVBs are critical for HBV assembly. During early endosomes mature towards late endosomes/MVBs, the formation of intraluminal vesicles is initiated, facilitated by the ESCRT machinery (Schmidt and Teis, 2012). ESCRT machinery comprises five main components known as ESCRT-0, –I, –II, –III, and vacuolar protein sorting 4 (VPS4) ATPase complex. These components act sequentially to complete vesicle abscission and release in cells. ESCRT-0, –I, and –II play pivotal roles in cargo recognition and membrane budding, subsequently recruiting ESCRT-III to catalyze the scission of membrane necks. The VPS4-mediated ATPase activity eventually disassembles the ESCRT complexes, allowing them recycling back to the cytoplasm (Hurley and Hanson, 2010). Notably, enveloped viruses exploit the ESCRT machinery for their assembly and secretion. While specific chemical inhibitors targeting the ESCRT pathway are lacking, siRNA and dominant mutation tools have provided valuable insights into the role of various ESCRT factors in the HBV life cycle. For instance, aberrant expression of the ESCRT-0 subunit HGS inhibits HBV replication, and overexpression of HGS promotes naked capsid release (Chou et al., 2015). Knockdown of ESCRT-I components TSG101 and Vps28 may in turn induce more functional ESCRT-II and III to assist virus release (Stieler and Prange, 2014). However, another project reported contrasting results. Zheng et al. showed evidence that TSG101 facilitates HBV assembly and egress by recognition of ubiquitylated HBc and delivery to MVB (Zheng Y. et al., 2023). Depletion of ESCRT-II components EAP20, EAP30, or EAP45 inhibits HBV production (Stieler and Prange, 2014). Overexpression of dominant negative mutant ESCRT-III subunits, like CHMP3 and CHMP4, also blocked HBV assembly and egress (Lambert et al., 2007). Additionally, VPS4 mutants have been shown to impede HBV replication and secretion without affecting S-HBsAg release (Kian Chua et al., 2006; Lambert et al., 2007). The secretion of filaments requires ESCRT-III and VPS4 (Jiang et al., 2015). However, loss of VPS4 function does not impair the level of secreted naked capsids (Watanabe et al., 2007), suggesting that HBV naked capsids and virions utilize diverse export pathways. A later study suggested Alix promotes capsid secretion independently of the ESCRT machinery but binds directly to the core via its Bro 1 domain, thereby highlighting an ESCRT-independent mechanism for HBV capsid export (Bardens et al., 2011). These studies have demonstrated the requirement of ESCRT machinery for HBV biogenesis: HBV components colocalize with ESCRT machinery and enter MVBs for their assembly; on the other hand, ESCRTs regulate MVB biogenesis and thereby modulate HBV assembly and release. The different roles of ESCRTs in HBV assembly and release indicate that more complicated mechanisms and interactions exist and need to be investigated further.

The mechanisms underlying how the HBV components access ESCRT and the processes of assembly within late endosomes/MVBs remain incompletely understood. A study identified a potential association

Fig. 1. The roles of autophagic and endosomal trafficking in the HBV lifecycle. The HBV infection cycle in hepatocytes includes receptor binding, entry, transport of capsids to the nuclear pore complex, covalently closed circular DNA (cccDNA) formation, transcription and translation, and assembly of capsids, viral particles, and subviral particles (SVPs). HBV enters host cells through clathrin-mediated endocytosis by interacting with the Na + -taurocholate co-transporting polypeptide (NTCP). HBsAg biosynthesis occurs at the endoplasmic reticulum (ER). HBsAg is subsequently transported to the pre-Golgi compartment, or to the early endosome and then released from host cells. The secretion process for HBV virions and filamentous SVPs depends on late endosome/multivesicular body (MVB)-associated endosomal sorting complex required for transport (ESCRT) machinery, which involves γ2-adaptin, α-taxilin, CHMP3, Nedd4, Alix, and Vps4. The abnormal buildup of HBsAg within the ER lumen induces ER stress and suppresses Akt/mTOR signaling, which, in turn, stimulates autophagy. The majority of autophagosomes normally undergo fusion with lysosomes for cargo degradation, assisted by Rab7. However, HBx intervenes by inhibiting the fusion of autophagosomes with lysosomes, thereby promoting HBV accumulation. Moreover, autophagosomes are also capable of fusing with late endosomes, forming amphisomes. HBV compartments accumulated in amphisomes can be released dependent on Rab11 and Rab27, indicating the autophagy-late endosome/MVB axis regulates HBV assembly and release.

between L-HBsAg and γ2-adaptin, facilitating their entry into late endosomes/MVBs (Lambert et al., 2007; Rost et al., 2006). Another reported adaptor may be α-taxilin, which interacts with the PreS1/PreS2 domain of LHBs and TSG101, thereby aiding in the recruitment of late endosomes/MVBs by envelope proteins (Hoffmann et al., 2013). HBV capsid may enter the ESCRT network by hijacking γ2-adaptin, the Nedd4 ubiquitin ligase, and TSG101, all of which were shown to interact with the viral core protein (Rost et al., 2006; Stieler and Prange, 2014; Zheng Y. et al., 2023). Given the significant involvement of late endosomes/MVBs in HBV maturation, progeny virions likely are released from cells similarly to exosome secretion or utilize exosomes as vehicles for release. Exosomes are extracellular vesicles (EVs) ranging from 30 to 200 nm in diameter that carry specific nucleic acids, proteins, and lipids (Pegtel and Gould, 2019). Prior investigations have detected HBV DNA, RNA, and HBV proteins in association with exosomes derived from HBV-infected hepatocytes (Jia et al., 2017; Wang J. et al., 2020). Further study from Eberhard's group has provided evidence for the presence of HBV markers on isolated exosomes, suggesting that intact HBV virions can be released as exosomes (Wu et al., 2023). Thus, HBV might exploit exosomes as a means of escape from cells. Considering that lipid rafts are present within endosomal membranes, inhibitors targeting lipid metabolism, which can impair MVBs biogenesis, exosome formation, and secretion may also influence virus replication and budding (Catalano and O'Driscoll, 2020). Our unpublished data shows that the inhibitors targeting lipid metabolism and exosome biogenesis, including pantethine, imipramine, and GW4869, impair HBV production, indicating that late endosomes/MVB-modulated HBV secretion may also be achieved by ESCRT-independent processes.

4. Autophagy and viral infection

Recently, autophagy is also found to efficiently support HBV replication at the steps of viral assembly and production (Chu et al., 2022; Lin et al., 2020b; Tian et al., 2011). Autophagy is necessary for maintaining cellular homeostasis by removing long-lived proteins and damaged organelles and recycling building blocks. It can be induced by various stimuli, including starvation, DNA damage, hypoxia, ER stress, and infection (Kroemer et al., 2010). In mammalian cells, phagophores are believed to primarily initiate from the ER (Yla-Anttila et al., 2009), and also possibly even derived from other cytosolic membrane structures, such as the ER-Golgi intermediate compartment (ERGIC) (Ge et al., 2013) and recycling endosomes (Puri et al., 2018). Phagophores expand and form double-membraned autophagosomes with sequestered cargo in. Subsequently, autophagosomes fuse with lysosomes, leading to the degradation of enclosed cargoes by lysosomal hydrolases (Mizushima, 2007). Autophagy serves multiple functions in mammalian cells and is

regarded as a potent defense mechanism against various viral infections. One of its roles involves restricting viral replication by delivering virus-related components to lysosomal degradation, thereby clearing the virus from the cell. For instance, SCOTIN, an IFN-β-inducible ER-resident protein (also known as SHISA5), facilitates the recruitment of HCV non-structural protein 5A (NS5A) to the autophagosome for degradation, effectively suppressing HCV replication (Kim et al., 2016). Similarly, Vif (viral infectivity factor), a key protein in human immunodeficiency virus 1 (HIV-1) infectivity and pathogenesis, is also subjected to autophagic degradation to control HIV-1 infectiveness (Valera et al., 2015). Furthermore, beyond its role in degradation, autophagy can also trigger innate immune responses. For example, vesicular stomatitis viruses (VSVs) are sensed in the lysosomes of plasmacytoid dendritic cells (pDCs) through TLR7, which are transported by means of autophagy after endocytosis of virions. In Atg5−/− pDCs, the production of IFN-α in response to VSV is impaired, indicating the role of autophagy in mediating pDC detection of ssRNA viruses and triggering IFN-α secretion (Lee et al., 2007). In reverse, some viruses require autophagic components for their own survival and replication, as their replication can be abolished by autophagy inhibitors (Choi et al., 2018). In our published work, we demonstrated that IFN-α triggered autophagy and promoted HBV replication in an *in-vitro* study (Li J. et al., 2022). Some viruses strategically exploit the early stages of autophagy and antagonize autophagosome maturation to prevent degradation (Kyei et al., 2009; Lin et al., 2020a), utilize autophagy-related lipid metabolism for efficient replication (Heaton and Randall, 2010; Kim et al., 2017), while some even exploit autophagy as an assembly platform and release pathway (Chu et al., 2022). Thus, autophagy plays an essential and dual-edged role in the viral life cycle and pathogenesis of viral infections.

4.1. Relation of autophagy and HBV

The exact role of autophagy in HBV replication is still under investigation. A recent systematic review about the interaction between HBV replication and cellular autophagy has summarized the available information (Lin et al., 2020b). HBV induces autophagy mainly through mechanisms involving hepatitis B x protein (HBx) and S-HBsAg. Specifically, HBx activates class III phosphatidylinositol 3-kinase (PI3KC3), mTOR, and AMP-activated protein kinase (AMPK) signaling pathways to enhance autophagosome formation (Bagga et al., 2016; Sir et al., 2010). On the other hand, S-HBsAg induces autophagy by triggering the unfolded protein response (UPR) and ER stress (Li et al., 2011).

HBV exploits the elements of the autophagy system for its envelopment. Li et al. reported that S-HBsAg co-localizes with autophagosomes and a blockage of autophagy significantly decreases extracellular virion secretion, providing evidence for the contribution of autophagosomes in viral envelop formation (Li et al., 2011). Prange's group demonstrated that HBV utilizes the ATG5-12-16L1 elongation complex as a physical scaffold for viral replication and envelopment (Doring et al., 2018). The association of ATG12 with the HBcAg/capsids has been experimentally proven, although pull-down experiments did not reveal a direct interaction between HBcAg and LC3B, implying that the co-localization of the HBcAg with LC3B may be mediated through ATG12 tethered to the phagophore. More recent evidence has shown that autophagic membrane-associated core particles are almost DNA replication competent (Chu et al., 2022).

Many factors like microRNAs (miRNAs), glucose, and other nutrients may influence the autophagic activity, thus modulating HBV replication. miRNAs are small, highly conserved noncoding RNAs known to play a role in regulating host-virus interactions. In the context of HBV infection, both upregulated and downregulated miRNAs have been identified in patients and cell models (Sartorius et al., 2019). Evidently, miRNAs are able to modulate various cellular processes including autophagy and thereby inhibit or enhance HBV replication. In hepatoma cells, the miR-99 family promotes HBV replication post-transcriptionally through IGF-1R/PI3K/Akt/mTOR/ULK1 signaling-induced autophagy (Lin et al., 2017), and miR-192-3p increases HBV replication through inhibiting ZNF143/Akt/mTOR signaling (Li F. et al., 2022). miR-146a-5p promotes autophagy and HBV replication via targeting the XIAP-mediated MDM2/p53 axis (Fu et al., 2019). Furthermore, mTOR acts as a sensor of energy status, regulating both autophagy and HBV replication, as detailed in our recent review (Wang et al., 2021). Similarly, AMPK is also an essential mediator of autophagy induction in response to energetic supplies (Wang S. et al., 2022). Low glucose concentration treatment leads to AMPK activation and triggers mTOR/ULK1/mediated autophagy, promoting HBV replication (Wang X. et al., 2020a) Decreased *O*-GlcNAcylation induces ER-stress, inhibits Akt/mTOR signaling, and blocks autophagosome-lysosome fusion, leading to an increased autophagy and enhanced HBV replication (Wang X, et al., 2020b). In contrast, the expression and export of HBeAg appear to be less or not dependent on autophagy. Consistent with our previous findings, the autophagic process and associated proteins do not seem to regulate HBV transcription or promoter activity, implying their involvement in the post-transcriptional steps of the HBV life cycle (Lin et al., 2017; Sir et al., 2010; Tian et al., 2011; Wang X. et al., 2020a).

4.2. Degradation of autophagosome-associated HBsAg and HBV virions by lysosomes

The lysosome is one of the final destinations of autophagosomes. While autophagy initiation is crucial for efficient HBV replication, many data also suggest that a substantial portion of HBsAg, capsid- and virion-associated HBV DNA undergo degradation by autophagy. Decreased fusion of autophagosomes with lysosomes increases HBV production. Rab7 is involved in the fusion of autophagosomes with lysosomes. Inhibiting Rab7 or knockdown of its effector PLEKHM1 prevents this step, thereby preserving the lysosomal degradation of HBV and causing strongly increased HBV yields (Lin et al., 2019a). Clearly, chloroquine (CQ), an agent that prevents the acidification of lysosomal compartments and autophagic degradation, exerts a similar effect (Lin et al., 2019a). Glucosamine, a derivative of glucose, shows the same effect as CQ (Lin et al., 2020a). The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, consisting of synaptosomal-associated protein 29 (SNAP29), vesicle-associated membrane protein 8 (VAMP8), and syntaxin 17 (STX17), plays a vital role in the autophagic degradation of HBV virions and SVPs by controlling autophagosome-lysosome fusion (Lin et al., 2019b). Liu et al. (2014) published data indicating that HBx helps HBV evade autophagic degradation by impairing lysosomal maturation. Moreover, interference with lipid metabolisms to modulate autophagosome-lysosome fusion also affects HBV replication. SAC1-like phosphatidylinositol phosphatase (SAC1), an integral membrane protein in the ER, plays a role in this process. Silencing SAC1 upregulates PI4P on the autophagosome membrane and blocks autophagy-lysosome fusion thereby promoting HBV replication (Zheng J. et al., 2023). Thus, efficient viral replication appears to rely on early autophagy while concurrently avoiding complete degradation. In contrast, autophagic membranes that successfully fuse with the lysosomes and complete lysosome enzymatic function are advantageous for controlling HBV replication. Treatment with epigallocatechin-3-gallate (EGCG) enhances lysosomal acidification and exhibits anti-HBV activity (Zhong et al., 2015). Additionally, promoting autolysosome-dependent degradation by overexpression of SAC1 and activation of AMPK inhibits HBV replication and production (Xie et al., 2016; Zheng J. et al., 2023). Therefore, gaining a better understanding of modulating autophagosome-lysosome fusion may be helpful to develop more efficient strategies to control HBV production. Recently, a host protein EVA1A has been shown to enhance HBV degradation and may play an active role in HBV control in patients with low viral loads (Yu et al., 2023).

5. Crosslink of autophagy and late endosome/MVB formation facilitates HBV particle secretion

ESCRTs have been shown related to autophagosome formation (Hurley, 2015). Rusten et al. summarized that ESCRT proteins control autophagy across four stages: inducing ER stress to initiate autophagy, facilitating phagophore closure, mediating fusion of autophagosomes with late endosomes/MVBs to form amphisome, and enabling fusion with lysosomes for degradation (Rusten and Stenmark, 2009). During the early steps, ATG12-ATG3 interacts with Alix to promote basal autophagic flux (Murrow et al., 2015). Takahashi et al. showed that the ESCRT-III components, CHMP2A, CHMP3, CHMP7, and VPS4A, are potential regulators of phagophore closure (Takahashi et al., 2018). Among these, CHMP2A translocating to the phagophore to regulate the separation of inner and outer membranes, ultimately forming double-membrane autophagosomes. The coordination between autophagic and endosomal pathways also regulates HBV assembly and secretion. While autophagy is traditionally viewed as an autodigestive pathway, it has been shown to also facilitate cellular secretion. Nevertheless, the mechanisms underlying "autophagic secretion" process remain unclear. In our latest study, we investigated the function of a protein named Gα-interacting vesicle-associated protein (GIV/Girdin). GIV was found to enhance HBV replication by increasing endosomal trafficking and reducing autophagic degradation of HBV proteins. Disrupting endocytosis by knockdown of GIV and its effectors led to the retention of HBsAg in ER and promotes autophagy and lysosomal degradation of HBV proteins. The results suggest that autophagy could promote HBV replication and production only when endosomal trafficking is at least partly functional (Wang et al., 2022b). Another study in our group found tunicamycin-induced ER stress and autophagic flux promoted HBV replication and the release of virions, SVPs, and naked capsids via the autophagosome-MVB axis (Wang et al., 2022a). Thus, the autophagic process is not merely a bypass of the endosomal pathway but rather a major pathway closely connected to late endosomes/MVBs for SVP and virion production. Amphisome, an intermediate organelle formed by the fusion of late endosomes with autophagosomes, is conventionally destined for degradation upon fusion with lysosomes (Zhao and Zhang, 2019). The regulation of amphisome formation and trafficking also affects HBV egress. Silencing Rab11, a small GTPase required for amphisome formation, leads to reduction of extracellular HBV DNA levels (Chu et al., 2022). Furthermore, our unpublished data also shows a blockade of late endosome/MVB formation enriched HBsAg in the amphisomes and limits the release of amphisome-involved HBV compartments. These observations underscore the importance of the intricate interplay between autophagy, endosomal trafficking, and HBV egress.

6. Conclusion and perspectives

In this review, we have summarized the interdependent and collaborative roles of the autophagic and endosomal pathways in HBV biogenesis. Early studies mainly focused on the direct effects of cellular host factors on HBV replication, often attempting to identify a molecular interaction of those factors with HBV components. Relevant cellular factors involved in endosomal membrane trafficking and autophagy have been extensively examined during the past years. Therefore, new approaches to understand the interaction of cellular factors and HBV need to be designed on the basis of our current knowledge. Nevertheless, numerous questions have arisen from the early studies and the current hypotheses, necessitating further exploration: how do relevant pathways cooperatively determine the distribution and export of HBV SVPs and virions and benefit for clinical practice? A better understanding of molecular mechanisms in two pathways would also be beneficial for development of new anti-HBV drugs. The production of mutated HBV components may disturb the cellular transport processes and result in cell death. Specific mutation in the preS/S gene may lead to the secretion defects of viral proteins and particles, resulting in an accumulation of viral products in the ER and trigger ER stress-associated autophagy and cause hepatocytes injury and may contribute to the pathogenesis of fulminant hepatitis (Wu et al., 2018). The roles of lysosomes in HBV production and degradation could be an interesting issue for future studies, for that it may be an important determinant of viral loads. Besides HBV infection, lysosomal changes and dysfunction have been correlated with the development of numerous human diseases, such as cancers, autoimmune disorders, and neurodegenerative diseases (Cao et al., 2021). Thus, drugs selectively targeting lysosomes and recovering the function could be a feasible strategy.

With the advancement of high-resolution microscopes, proteomic, and genomic screening techniques, we will acquire more comprehensive information and a deeper understanding of the molecular mechanisms underlying endosomal and autophagic vesicle pathways and HBV biogenesis. We can look forward to developing excellent therapeutic intervention by modulating the pathways.

Conflict of interest

Prof. Mengji Lu is an editorial board member for *Virologica Sinica* and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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