

MICROREVIEW

WILEY

Hepatitis E virus egress and beyond – the manifold roles of the viral ORF3 protein

Mirco Glitscher  | Eberhard Hildt 

Department Virology, Paul-Ehrlich-Institut, Langen, Germany

Correspondence

Eberhard Hildt, Department Virology, Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 51-59, D-63225 Langen, Germany.
Email: eberhard.hildt@pei.de

Funding information

LOEWE Center DRUID (Novel Drug Targets against Poverty-Related and Neglected Tropical Infectious Diseases; project D2)

Abstract

Although the hepatitis E virus represents an uprising threat to the global community by representing the commonest cause of an acute viral hepatitis worldwide, its life cycle is grossly understudied. Albeit HEV is a non-enveloped virus, its progeny is released as quasi-enveloped virions. Thus, the responsible accessory protein pORF3 gained rising attention in the past years. It mediates viral release via the exosomal route by targeting the viral capsid to the endosomal system, more precisely to multivesicular bodies. As this is followed by quasi-envelopment, pORF3 may in terms represent a substitute to a conventional envelope protein. This feature proves to be rather unique with respect to other enteric viruses, although the protein's role in the viral life cycle seems to reach far beyond simply maintaining release of progeny viruses. How pORF3 affects viral morphogenesis, how it mediates efficient viral release and how it supports viral spread is summarised in this microreview. With this, we aim to shed light on functions of pORF3 to gain further insights in still enigmatic aspects of the HEV life cycle.

Take Aways

- HEV is released as exosome via multivesicular bodies
- Viral pORF3 mediates release via endosomal complexes required for transport
- pORF3 modulates various cellular processes in infected cells
- Elucidation of pORF3-related processes imply novel clinical strategies

KEYWORDS

exosome, HEV, MVB

1 | THE HEPATITIS E VIRUS – GENOMIC ORGANISATION

The hepatitis E virus (HEV) is the sole member of the genus *Orthohepevirus* in the *Hepeviridae* family. It represents a non-enveloped, positive-sense, single-stranded RNA virus with a genome being 7.2–7.4 kb in size (Tam et al., 1991). Three open reading frames (ORFs) are encoded by all genotypes, yet only HEV genotype 1 carries a fourth ORF

(Nair et al., 2016). Proteins encoded by ORF1 and ORF4 are initially translated from the genome and mediate genomic replication (Koonin et al., 1992). Whether pORF1 is proteolytically cleaved or acts as single polyprotein remains elusive, yet both the full-length protein and separately expressed subunits display functionality (Parvez, 2017). During this process, an antisense RNA is synthesised (Nanda, Panda, Durgapal, & Jameel, 1994) serving as template for both the viral genome and a 2.2 kb bicistronic subgenomic RNA comprising ORF2 and ORF3 (Graff, Torian,

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *Cellular Microbiology* published by John Wiley & Sons Ltd.

Nguyen, & Emerson, 2006). The positive-sense transcripts are 5'-capped and 3'-polyadenylated (Kabrane-Lazizi, Meng, Purcell, & Emerson, 1999). Following the cap-structure lies a 5'-UTR, whereas a 3'-UTR precedes the genomically encoded polyadenylation. Both UTRs found in the genomic RNA carry regulatory RNA secondary structures or cis-responsive elements (CREs). A third CRE is found within the region separating ORF1

and the overlapping ORF2/3 (Ju et al., 2020). This CRE-mediated separation of the viral genome potentially serves regulatory purposes for initializing and terminating subgenomic RNA synthesis and therefore impacts translation of the proteins encoded by ORF2 (pORF2) and ORF3 (pORF3) (Ding et al., 2018) (Figure 1a). These in turn are the drivers of viral morphogenesis and release.

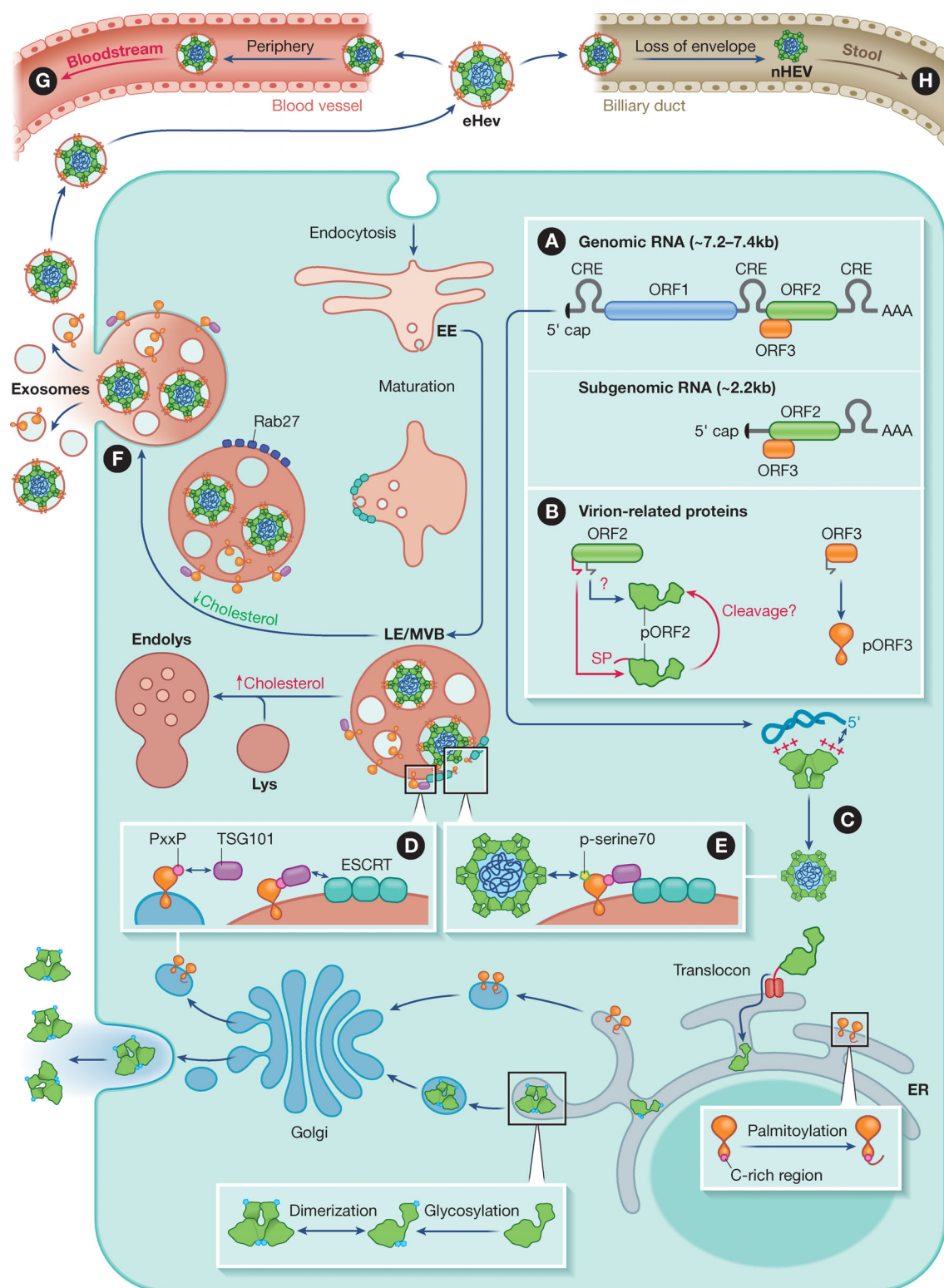


FIGURE 1 Legend on next page.

2 | HEV – SHOULD WE CARE?

The *Hepeviridae* family comprises eight genotypes within the species *Orthohepevirus A* (Smith et al., 2014). While genotypes 1 and 2 are restricted to humans, genotypes 3, 4 and 7 display zoonotic potential (Doceul, Bagdassarian, Demange, & Pavio, 2016; Sridhar, Teng, Chiu, Lau, & Woo, 2017). Thus, transmission not only occurs via contaminated sewage or blood-products (Colson et al., 2007; Viswanathan, 2013), but also via contaminated meat (Harrison & DiCaprio, 2018). HEV represents the commonest cause of an acute viral hepatitis worldwide. As of 2005, there are over 20 million newly registered cases per year, resulting in 3 million symptomatic cases and 70,000 related fatalities (Rein, Stevens, Theaker, Wittenborn, & Wiersma, 2012). However, these numbers have risen up until today, suggesting an even higher death toll (P. Li et al., 2020). Herein, mortality rates range from 0.1 to 4% in healthy adults to up to ~30% in pregnant women (Jilani et al., 2007). Increased mortality in the latter is due to the fulminant course the disease can take in pregnant women infected with genotype 1 or 2 (Gouilly et al., 2018). Immunosuppressed patients represent a further risk group for they often develop a chronic hepatitis upon infection with genotype 3 or 4 (Kamar et al., 2008). This further links the course of disease to the geographical spread, as zoonotic genotypes majorly reside in industrialised nations, yet human-only genotypes are endemic predominantly in emergent nations (Aggarwal, 2011). As per disease-prevention, a vaccine is only approved in China (S.-W. Li et al., 2015), and treatment options are limited. Here, PEGylated interferon alpha (Kamar, Abravanel, et al., 2010) and majorly ribavirin (Kamar, Rostaing, et al., 2010) are used, yet both cause severe adverse effects (Manns, Wedemeyer, & Cornberg, 2006; Yang et al., 2010), and the latter may confer drug resistance due to viral mutation (Debing et al., 2014). In essence, HEV represents a pathogen being on the rise from a global perspective. There is a tremendous lack in disease prevention and management due to a

lack of understanding the viral life cycle. Thus, elucidating the release and spread of progeny viruses is of central interest.

3 | THE VIRAL CAPSID

The viral capsid protein is represented by pORF2 and is 660 amino acids (aa) in length. It can be separated into three domains: (a) an aminoterminal (N-terminal) shell domain (S), (b) a middle domain (M) and a carboxyterminal (C-terminal) protrusion domain (P) (Yamashita et al., 2009). Further, the full-length protein carries a signal peptide (Figure 1b), which thereby renders localization to the endoplasmic reticulum (ER). Therein, pORF2 is glycosylated at two asparagine residues being part of consensus N-X-S/T motifs (Ankavay et al., 2019). While a study implied that this post-translational modification may fulfil virion-related roles (Graff et al., 2008), a more recent study pointed out that there in fact exist three different forms of the capsid protein. While the glycosylated forms are secreted as dimers only, the unglycosylated form is responsible for forming the viral capsid. Reason for a lack in glycosylation may either be found by translation via a downstream shifted start-codon or by an N-terminal cleavage, which is yet to be clarified in more detail (Montpellier et al., 2018; Yin et al., 2018). Assembly of the capsid structure is mediated by spontaneous homodimerisation within the P-domains near the C-terminal end (Xu, Behloul, Wen, Zhang, & Meng, 2016) and subsequent oligomerisation (T.-C. Li et al., 2005). During this process, 5'-end-dependent RNA-binding and encapsidation is mediated via an N-terminal, positively charged stretch within the S-domain (Surjit, Jameel, & Lal, 2004) (Figure 1c). While the site of RNA encapsidation requires further elucidation, an involvement of the ER and the cis- and trans-Golgi-network was suggested in bringing components into close proximity of each other (Nagashima, Takahashi, et al., 2014; Surjit, Jameel, & Lal, 2007). As a result, an icosahedral capsid structure is formed, which likely reflects a T = 3 symmetry for RNA-loaded particles (Xing et al., 2010). These

FIGURE 1 HEV pORF3 mediates essential processes during viral release. The 5'-capped and 3'-polyadenylated viral genome comprises three ORFs, which are flanked by stem-loop structures forming CREs. ORF2 and ORF3 are contained within a similarly modified bicistronic subgenomic RNA (a). ORF2 encodes the capsid protein pORF2 containing an SP in its full form, yet no SP if either an alternate start-codon is used or N-terminal cleavage occurs. ORF3 encodes a virion-related regulatory protein pORF3 (b). The SP-containing form of pORF2 is imported into the ER and is therein glycosylated and dimerizes. Subsequently, glycosylated pORF2 dimers shuttle from ER through the Golgi apparatus and are secreted. In contrast, the non-glycosylated pORF2 dimerizes and binds the viral genome at the 5'-end. The interaction is stabilised via a positively charged stretch within pORF2 and leads to RNA encapsidation (c). pORF3 in turn represents either an integral membrane protein or a peripheral membrane protein being palmitoylated at a cysteine-rich region. pORF3 then locates to MVBs where it interacts with TSG101 via a PxxP motif. This subsequently leads to translocation of pORF3 to the ESCRT machinery (d). Subsequently, HEV nucleocapsids interact with phosphorylated pORF3 at the surface of MVBs, which triggers invagination as ILVs and therefore viral quasi-envelope (e). HEV-loaded MVBs then shuttle to the PM in a Rab27-dependent manner once there are low intracellular cholesterol concentrations. High concentrations of the lipid induce lysosomal degradation of the viral cargo. Upon MVB-PM fusion, quasi-enveloped viral particles are released (f). These viral particles maintain their quasi envelope within the bloodstream of infected patients (g). Just upon entry into the biliary duct, bile acids lead to removal of the quasi-envelope and expose the nucleocapsid. Subsequently, shedding of viral particles takes place via stool (h). AAA, polyadenylation; C-rich region, cysteine-rich region; CRE, cis-responsive element; EE, early endosome; eHEV, quasi-enveloped HEV virions; Endolys, endolysosome; ER, endoplasmic reticulum; LE/MVB, late endosome/multivesicular body; Lys, lysosome; nHEV, non-envelope HEV virions; ORF, open reading frame; p-serine70, phosphorylated serine-residue at position 70; pORF, protein encoded by ORF; PSAP, PSAP motif; SP, signal peptide

virions can be detected in stool samples acquired from infected patients, which display a size of ~27 nm (Balayan et al., 1983).

4 | THE ROLE OF pORF3 IN VIRIONS

Despite HEV virions appearing as non-enveloped in stool samples with a distinctive size, further studies described the presence of larger particles in cell culture supernatants and blood samples. Here, HEV particles were characterised with a size of ~40 nm ranging up to 120 nm (Montpellier et al., 2018; Nagashima, Jirintai, et al., 2014). Along with changes in size went changes in particle density, which range from ~1.07 to 1.15 g/ml in case of blood- and cell culture-derived particles to ~1.21–1.28 g/ml for stool-derived virions. This change is reasoned by these particles carrying a lipid envelope surrounding the capsid structure (Emerson et al., 2010; Qi et al., 2015; Takahashi et al., 2010). This primed investigations regarding the characterisation of the two forms of virions: non-enveloped HEV (nHEV) and quasi-enveloped HEV (eHEV). A central role of the viral protein pORF3 became apparent herein, which seems to be dispensable for the entirety of viral life cycle except for viral egress (Emerson et al., 2010; Emerson, Nguyen, Torian, & Purcell, 2006; Yamada et al., 2009). The protein is ~113 aa in size and resides within the viral quasi-envelope (Takahashi et al., 2008; Takahashi et al., 2016). The exact cause for pORF3 being membrane-associated may either be caused by a predicted transmembrane domain (TMD) (Ding et al., 2017) or via palmitoylation at an N-terminal cysteine-rich region (Gouttenoire et al., 2018), which is yet to be clarified. As further modification, a phosphorylation by host-kinases cyclin-dependent kinase 1 (CDK1) or mitogen-activated protein kinases (MAPKs) at a serine residue on position 70 was described (Zafrullah, Ozdener, Panda, & Jameel, 1997). This directly lies within a region located between aa 57–80, which is described to be crucial for interaction with non-glycosylated pORF2 and thereby rendering a phosphorylation-dependent interaction between pORF3 and nHEV (Tyagi, Korkaya, Zafrullah, Jameel, & Lal, 2002) (Figure 1e). Although following spatio-temporal relationships remain elusive in large parts, a microtubule association of pORF3 mediated via its N-terminal hydrophobic domains (Kannan, Fan, Patel, Bossis, & Zhang, 2009; Zafrullah et al., 1997) may potentially serve purposes of trafficking the capsid to its destination or mediating contact to downstream organelles. In effect, the covering of capsids with pORF3 primes endosomal targeting, which is followed by viral quasi-envelopment.

5 | ENVELOPMENT AND VIRAL EGRESS

Central key players within the endosomal system are multivesicular bodies (MVBs). These represent a specified subset of late endosomes, which are characterised by a distinct set of proteins, lipids and accumulated intraluminal vesicles (ILVs). The build-up and sorting into ILVs hereby determines the fate of bound cargo with respect to degradation, subcellular translocation or secretion. ILVs are formed by the

MVB membrane-resident endosomal sorting complexes required for transport (ESCRT), which are responsible for cargo recognition and sorting (ESCRT-0 and -I), cargo condensation (ESCRT-II) and budding of vesicles from the MVB surface into the lumen (ESCRT-III) (Rodriguez-Furlan, Minina, & Hicks, 2019). For HEV, MVBs and the ESCRT machinery are essential for particle quasi-envelopment, which is initiated by pORF3-mediating interaction with the host-factor tumour susceptibility gene 101 (TSG101) (Nagashima, Takahashi, Jirintai, Tanaka, Yamada, et al., 2011). The latter is an MVB-resident protein and part of ESCRT-I, thus being involved in cargo- recognition and sorting (Sundquist et al., 2004). An interaction between HEV and this host factor is dependent on a viral late-domain located in pORF3 upstream of the pORF2 interacting domain, where in human pathogenic species of HEV a classical PSAP motif can be found at position 96–99 close to the C-terminus (Emerson et al., 2010) (Figure 1d). Effectively, this leads to a recruitment of capsid structures to the site of ESCRT-mediated formation of ILVs. As a consequence, viral capsids enter MVBs as ILVs, which then leads to endosomal shuttling towards the plasma membrane (PM) in a Ras-related protein 27a (Rab27a)-dependent manner (Nagashima et al., 2017) (Figure 1f). Efficient shuttling and escape from endosomal-lysosomal fusion herein seem to be dependent on cholesterol. Specifically, high cholesterol levels shift the equilibrium of the endosomal flux towards degradative processes, yet withdrawal of cholesterol favours successful release (Glitscher et al., 2021). Ultimately, viral release is achieved by HEV-containing MVBs fusing with the PM. Predominantly, this is suggested to take place at the apical interface of polarised hepatocytes (Capelli et al., 2019), which may be reasoned by pORF3 being found predominantly at the apical interface of polarised cells. This in turn may highlight its role in efficiently mediating release towards the biliary duct to guarantee viral shedding. As virions ultimately are released via MVBs, the viral quasi-envelope comprises host factors such as ALG-2-interacting Protein X (ALIX), vacuolar protein sorting-associated protein 4A (Vps4A), Vps4B, trans-Golgi network integral membrane protein 2 (TGOLN2) or tetraspanins, which are characteristic markers for classical exosomes (Nagashima, Takahashi, et al., 2014; Nagashima et al., 2017; Primadharsini et al., 2020) alongside carrying exosome-specific lipids (Chapuy-Regaud et al., 2017) while circulating in the bloodstream (Figure 1g). Just upon entry into the biliary duct, the virions are believed to lose their membranous shell due to the presence of detergents within the bile, which goes along with a tenfold increase in infectivity (Yin, Ambardekar, Lu, & Feng, 2016) and viral shedding into the stool (Figure 1h). This route of egress represents the major mechanism of viral release for HEV, which is highlighted by interference with endosomal/exosomal maturation or pORF3 functionality leading to a loss in productive progeny formation (Anang et al., 2018; Kenney, Wentworth, Heffron, & Meng, 2015; Nagashima, Takahashi, Jirintai, Tanaka, Nishizawa, et al., 2011; Nagashima, Jirintai, et al., 2014; Tanggis et al., 2018). Notably, the importance of pORF3 for this process and its role for subsequent particle production seems to be conserved over different hosts and species of HEV, although alternate late-domain sequences may be in place (Kenney et al., 2012; Primadharsini et al., 2020). Thus, the exosomal release and acquisition of a quasi-

envelope around the otherwise non-enveloped virions of HEV is entirely dependent on pORF3.

6 | pORF3 MAKING THE DIFFERENCE

Various different viruses were described to use MVBs and the ESCRT machinery during their viral life cycle or for release of viral by-

products. Among these are other hepatotropic viruses such as the hepatitis B virus (HBV) (Hoffmann et al., 2013; B. Jiang, Himmelsbach, Ren, Boller, & Hildt, 2015; Lambert, Döring, & Prange, 2007), the hepatitis C virus (HCV) (Elgner et al., 2016; Masciopinto et al., 2004) or the hepatitis A virus (HAV) (Feng et al., 2013). What makes both HAV and HEV unique in comparison to HBV and HCV is that both normally form non-enveloped virions as they encode no envelope proteins. Conventionally, non-enveloped viruses were believed to be released

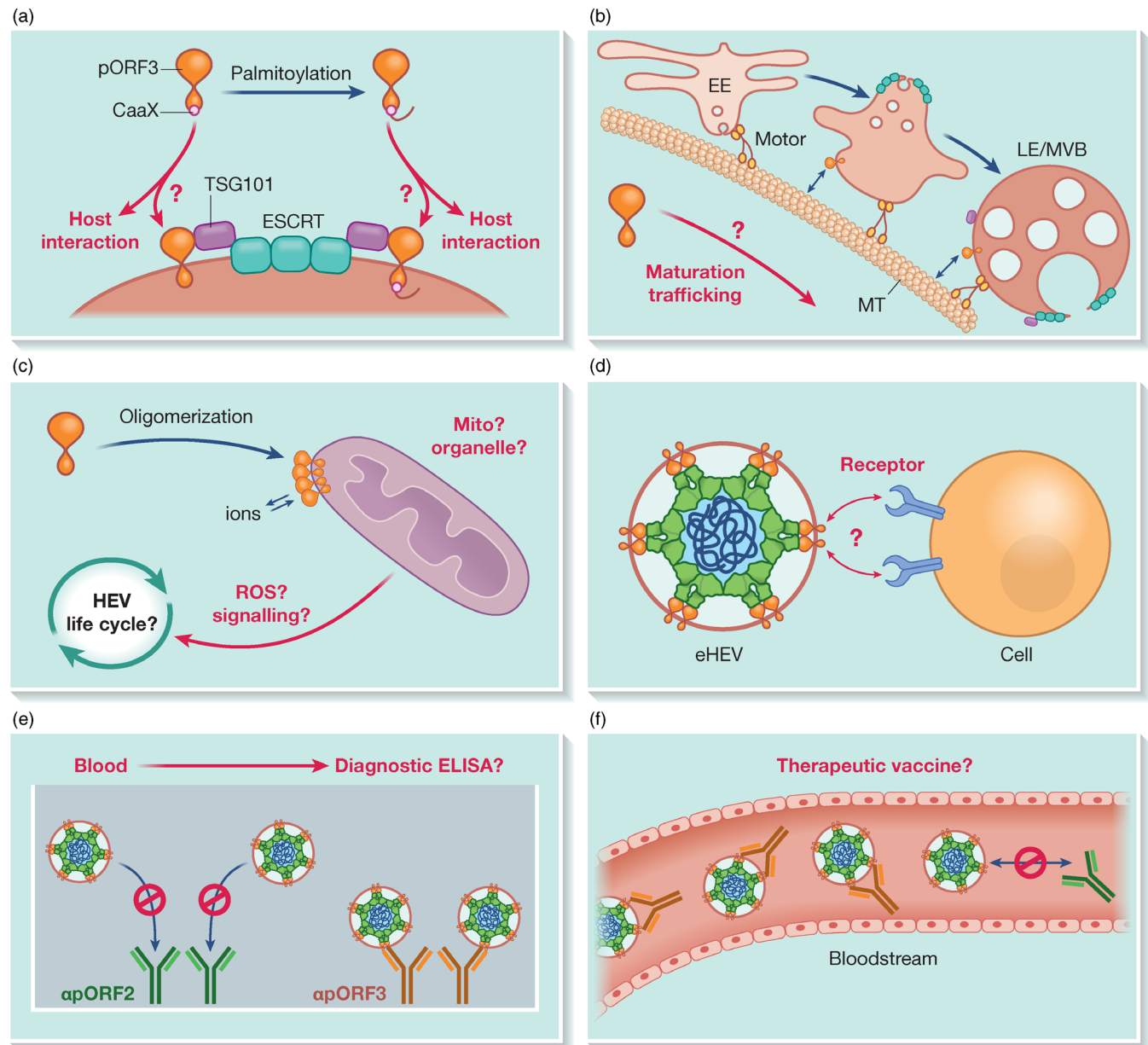


FIGURE 2 Manifold roles of HEV pORF3 open various questions and applications. (a) Which form of pORF3, the palmitoylated or the non-modified, is predominantly found associated with the ESCRT machinery remains elusive. Further host-protein interactions and related cellular processes require clarification. (b) The MT association of pORF3 may influence endosomal maturation and trafficking. (c) Channel activity mediated via pORF3 oligomerization potentially leads to altered functions of mitochondria and other organelles. This in turn would influence the viral life cycle. (d) As pORF3 is partially exposed on the surface of eHEV, an alternate receptor may be in place for viral entry. (e) Diagnostic tools targeting pORF3 may help to determine viral titers in blood samples. (f) The presence of pORF3 on eHEV may prove useful as a strategy for a therapeutic vaccine targeting the viral spread within an organism. EE, early endosome; eHEV, quasi-enveloped HEV virions; LE/MVB, late endosome/multivesicular body; Mito, mitochondrion; MT, microtubule; pORF, protein encoded by ORF; ROS, reactive oxygen species

strictly via entering a lytic cycle, which in fact was proven untrue and gained increasing interest in the past decade especially for enteric viruses (Owusu, Quaye, Passalacqua, & Wobus, 2021). With respect to this, HAV and HEV have adapted strikingly similar mechanisms corroborating the exosomal route of egress (Feng, Hirai-Yuki, McKnight, & Lemon, 2014). Nonetheless, while HAV enters MVBs directly via its capsid (González-López et al., 2018; W. Jiang et al., 2020), HEV differs insofar as that it dedicated a separate protein for this purpose: pORF3. Due to its membrane localization and interaction with the capsid structure, it features aspects of classical envelope proteins. In this context, the question whether pORF3 represents an integral or a peripheral membrane protein, as suggested via abrogation of palmitoylation (Gouttenoire et al., 2018), needs to be addressed in more detail. Further, the mechanism compromised by pORF3 presents rather reminiscent of the Gag p6-protein of human immunodeficiency virus (HIV), which similarly targets TSG101 via a PxxP-motif (Garrus et al., 2001) and of HBV making use of the host-factor α -taxilin (TXLNA) to initiate TSG101-interaction (Hoffmann et al., 2013). Thus, analysing further host–pathogen interaction potentially being hijacked by HEV may prove useful for additional understanding (Figure 2a,b). Alongside this direct interaction with the exosomal pathway, regulatory roles of pORF3 herein require further attention. Evidently, the viral protein modulates mitochondrial processes (Moin, Panteva, & Jameel, 2007; Tian et al., 2019), possibly via acting as ion channel (Ding et al., 2017). In how far these mechanisms contribute to beneficial viral release, for example, via induction of elevated levels of reactive oxygen species, as described for HCV (Medvedev et al., 2017), presents an issue worth elucidating (Figure 2c). Additionally, pORF3 is described to interfere with cellular host defences such as the inducibility as well as the effectors of interferon signalling (Dong et al., 2012; He et al., 2016; Lei et al., 2018). This in turn further helps the viral infection to be efficiently established. Conclusively, HEV pORF3 may in terms be regarded as an alternate envelope protein of a quasi-enveloped virus, which is partially presented on the surface of eHEV (Qi et al., 2015; Takahashi et al., 2008; Takahashi et al., 2010). In this context, a potential role of pORF3 in viral entry needs to be clarified in more detail, as different routes of viral entry are described for eHEV and nHEV (Yin et al., 2016) (Figure 2d). Knowledge gathered herein will also serve purposes of pinpointing risks of extrahepatic manifestations. From a clinical point of view, the incorporation of pORF3 into eHEV virions also raises questions with respect to diagnostics and disease prevention. First, establishment of ELISA-based assays recognising pORF3 may prove useful to determine actual viral loads in infected patients (Figure 2e). Problems in current assays targeting pORF2 are both shielding of antigens via the quasi-envelope and secretion of pORF2 dimers, which may deteriorate readouts depending on the question being addressed. Further, pORF3 may as well be used as a target for therapeutic vaccinations in addition to the pORF2-based vaccine as experimentally implemented before, for example, in chicken (Syed et al., 2017). As such, it may inactivate eHEV once the virus is in circulation and may in this context be superior to the current pORF2-based vaccine (Figure 2f). In summary, HEV pORF3 represents

a small yet central viral protein being of utmost importance for the viral life cycle. As such, it regulates fundamental cellular processes via various different modes of action. Especially its involvement in the unconventional viral egress makes it a target worth being studied, which may help to overcome current gaps in knowledge about HEV and the related lack in options for disease management.

ACKNOWLEDGEMENT

This work was supported with a grant by the LOEWE Center DRUID (Novel Drug Targets against Poverty-Related and Neglected Tropical Infectious Diseases; project D2). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Eberhard Hildt and Mirco Glitscher: Conceptualisation. **Mirco Glitscher:** Writing – original draft preparation. **Eberhard Hildt:** Writing – review and editing. **Mirco Glitscher:** Visualisation. **Eberhard Hildt:** Supervision. **Eberhard Hildt:** Project administration. **Eberhard Hildt:** Funding acquisition.

DATA AVAILABILITY STATEMENT

Data sharing not applicable – no new data generated, or the article describes entirely theoretical research.

ORCID

Mirco Glitscher  <https://orcid.org/0000-0002-8799-7565>

Eberhard Hildt  <https://orcid.org/0000-0002-3020-9564>

REFERENCES

- Aggarwal, R. (2011). Hepatitis E: Historical, contemporary and future perspectives. *Journal of Gastroenterology and Hepatology*, 26(Suppl 1), 72–82.
- Anang, S., Kaushik, N., Hingane, S., Kumari, A., Gupta, J., Asthana, S., ... Surjit, M. (2018). Potent inhibition of hepatitis E virus release by a cyclic peptide inhibitor of the interaction between viral open reading frame 3 protein and host tumor susceptibility gene 101. *Journal of Virology*, 92(20), e00684–18.
- Ankavay, M., Montpellier, C., Sayed, I. M., Saliou, J.-M., Wychowski, C., Saas, L., ... Cocquerel, L. (2019). New insights into the ORF2 capsid protein, a key player of the hepatitis E virus lifecycle. *Scientific Reports*, 9(1), 6243.
- Balayan, M. S., Andjaparidze, A. G., Savinskaya, S. S., Ketiladze, E. S., Braginsky, D. M., Savinov, A. P., & Poleschuk, V. F. (1983). Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology*, 20(1), 23–31.
- Capelli, N., Marion, O., Dubois, M., Allart, S., Bertrand-Michel, J., Lhomme, S., ... Chapuy-Regaud, S. (2019). Vectorial release of hepatitis E virus in polarized human hepatocytes. *Journal of Virology*, 93(4), e01207–18.
- Chapuy-Regaud, S., Dubois, M., Plisson-Chastang, C., Bonnefois, T., Lhomme, S., Bertrand-Michel, J., ... Izopet, J. (2017). Characterization of the lipid envelope of exosome encapsulated HEV particles protected from the immune response. *Biochimie*, 141, 70–79.
- Colson, P., Coze, C., Gallian, P., Henry, M., de Micco, P., & Tamalet, C. (2007). Transfusion-associated hepatitis E, France. *Emerging Infectious Diseases*, 13(4), 648–649.

- Debing, Y., Gisa, A., Dallmeier, K., Pischke, S., Bremer, B., Manns, M., ... Neyts, J. (2014). A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. *Gastroenterology*, 147(5), 1008–11.e7; quiz e15–6.
- Ding, Q., Heller, B., Capuccino, J. M. V., Song, B., Nimgaonkar, I., Hrebikova, G., ... Ploss, A. (2017). Hepatitis E virus ORF3 is a functional ion channel required for release of infectious particles. *Proceedings of the National Academy of Sciences of the United States of America*, 114(5), 1147–1152.
- Ding, Q., Nimgaonkar, I., Archer, N. F., Bram, Y., Heller, B., Schwartz, R. E., & Ploss, A. (2018). Identification of the Intragenomic promoter controlling hepatitis E virus subgenomic RNA transcription. *mBio*, 9(3), e00769–18.
- Doceul, V., Bagdassarian, E., Demange, A., & Pavio, N. (2016). Zoonotic hepatitis E virus: Classification, animal reservoirs and transmission routes. *Viruses*, 8(10), 270.
- Dong, C., Zafrullah, M., Mixson-Hayden, T., Dai, X., Liang, J., Meng, J., & Kamili, S. (2012). Suppression of interferon- α signaling by hepatitis E virus. *Hepatology*, 55(5), 1324–1332.
- Elgner, F., Ren, H., Medvedev, R., Ploen, D., Himmelsbach, K., Boller, K., & Hildt, E. (2016). The intracellular cholesterol transport inhibitor U18666A inhibits the exosome-dependent release of mature hepatitis C virus. *Journal of Virology*, 90(24), 11181–11196.
- Emerson, S. U., Nguyen, H., Torian, U., & Purcell, R. H. (2006). ORF3 protein of hepatitis E virus is not required for replication, virion assembly, or infection of hepatoma cells in vitro. *Journal of Virology*, 80(21), 10457–10464.
- Emerson, S. U., Nguyen, H. T., Torian, U., Burke, D., Engle, R., & Purcell, R. H. (2010). Release of genotype 1 hepatitis E virus from cultured hepatoma and polarized intestinal cells depends on open reading frame 3 protein and requires an intact PXXP motif. *Journal of Virology*, 84(18), 9059–9069.
- Feng, Z., Hensley, L., McKnight, K. L., Hu, F., Madden, V., Ping, L., ... Lemon, S. M. (2013). A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. *Nature*, 496(7445), 367–371.
- Feng, Z., Hirai-Yuki, A., McKnight, K. L., & Lemon, S. M. (2014). Naked viruses that aren't always naked: Quasi-enveloped agents of acute hepatitis. *Annual Review of Virology*, 1(1), 539–560.
- Garrus, J. E., von Schwedler, U. K., Pornillos, O. W., Morham, S. G., Zavitz, K. H., Wang, H. E., ... Sundquist, W. I. (2001). Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell*, 107(1), 55–65.
- Glitscher, M., Martín, D. H., Woytinek, K., Schmidt, B., Tabari, D., Scholl, C., ... Hildt, E. (2021). Targeting cholesterol metabolism as efficient antiviral strategy against the hepatitis E virus. *Cellular and Molecular Gastroenterology and Hepatology*, 12, 159–180.
- González-López, O., Rivera-Serrano, E. E., Hu, F., Hensley, L., McKnight, K. L., Ren, J., ... Lemon, S. M. (2018). Redundant late domain functions of tandem VP2 YPX₃L motifs in nonlytic cellular egress of quasi-enveloped hepatitis A virus. *Journal of Virology*, 92(23), e01308–18.
- Gouilly, J., Chen, Q., Siewiera, J., Cartron, G., Levy, C., Dubois, M., ... el Costa, H. (2018). Genotype specific pathogenicity of hepatitis E virus at the human maternal-fetal interface. *Nature Communications*, 9(1), 4748.
- Gouttenoire, J., Pollán, A., Abrami, L., Oechslin, N., Mauron, J., Matter, M., ... Moradpour, D. (2018). Palmitoylation mediates membrane association of hepatitis E virus ORF3 protein and is required for infectious particle secretion. *PLoS Pathogens*, 14(12), e1007471.
- Graff, J., Torian, U., Nguyen, H., & Emerson, S. U. (2006). A bicistronic subgenomic mRNA encodes both the ORF2 and ORF3 proteins of hepatitis E virus. *Journal of Virology*, 80(12), 5919–5926.
- Graff, J., Zhou, Y.-H., Torian, U., Nguyen, H., St Claire, M., Yu, C., ... Emerson, S. U. (2008). Mutations within potential glycosylation sites in the capsid protein of hepatitis E virus prevent the formation of infectious virus particles. *Journal of Virology*, 82(3), 1185–1194.
- Harrison, L., & DiCaprio, E. (2018). Hepatitis E virus: An emerging foodborne pathogen. *Frontiers in Sustainable Food Systems*, 2, 14.
- He, M., Wang, M., Huang, Y., Peng, W., Zheng, Z., Xia, N., ... Tian, D. (2016). The ORF3 protein of genotype 1 hepatitis E virus suppresses TLR3-induced NF- κ B signaling via TRADD and RIP1. *Scientific Reports*, 6, 27597.
- Hoffmann, J., Boehm, C., Himmelsbach, K., Donnerhak, C., Roettger, H., Weiss, T. S., ... Hildt, E. (2013). Identification of α -taxilin as an essential factor for the life cycle of hepatitis B virus. *Journal of Hepatology*, 59(5), 934–941.
- Jiang, B., Himmelsbach, K., Ren, H., Boller, K., & Hildt, E. (2015). Subviral hepatitis B virus filaments, like infectious viral particles, are released via multivesicular bodies. *Journal of Virology*, 90(7), 3330–3341.
- Jiang, W., Ma, P., Deng, L., Liu, Z., Wang, X., Liu, X., & Long, G. (2020). Hepatitis A virus structural protein pX interacts with ALIX and promotes the secretion of virions and foreign proteins through exosome-like vesicles. *J Extracell Vesicles*, 9(1), 1716513.
- Jilani, N., Das, B. C., Husain, S. A., Baweja, U. K., Chattopadhyay, D., Gupta, R. K., ... Kar, P. (2007). Hepatitis E virus infection and fulminant hepatic failure during pregnancy. *Journal of Gastroenterology and Hepatology*, 22(5), 676–682.
- Ju, X., Xiang, G., Gong, M., Yang, R., Qin, J., Li, Y., ... Ding, Q. (2020). Identification of functional cis-acting RNA elements in the hepatitis E virus genome required for viral replication. *PLoS Pathogens*, 16(5), e1008488.
- Kabrane-Lazizi, Y., Meng, X. J., Purcell, R. H., & Emerson, S. U. (1999). Evidence that the genomic RNA of hepatitis E virus is capped. *Journal of Virology*, 73(10), 8848–8850.
- Kamar, N., Abravanel, F., Garrouste, C., Cardeau-Desangles, I., Mansuy, J. M., Weclawiak, H., ... Rostaing, L. (2010). Three-month pegylated interferon-alpha-2a therapy for chronic hepatitis E virus infection in a haemodialysis patient. *Nephrology, Dialysis, Transplantation*, 25(8), 2792–2795.
- Kamar, N., Rostaing, L., Abravanel, F., Garrouste, C., Lhomme, S., Esposito, L., ... Izopet, J. (2010). Ribavirin therapy inhibits viral replication on patients with chronic hepatitis E virus infection. *Gastroenterology*, 139(5), 1612–1618.
- Kamar, N., Selves, J., Mansuy, J.-M., Ouezzani, L., Péron, J.-M., Guitard, J., ... Rostaing, L. (2008). Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *The New England Journal of Medicine*, 358(8), 811–817.
- Kannan, H., Fan, S., Patel, D., Bossis, I., & Zhang, Y.-J. (2009). The hepatitis E virus open reading frame 3 product interacts with microtubules and interferes with their dynamics. *Journal of Virology*, 83(13), 6375–6382.
- Kenney, S. P., Pudupakam, R. S., Huang, Y.-W., Pierson, F. W., LeRoith, T., & Meng, X.-J. (2012). The PSAP motif within the ORF3 protein of an avian strain of the hepatitis E virus is not critical for viral infectivity in vivo but plays a role in virus release. *Journal of Virology*, 86(10), 5637–5646.
- Kenney, S. P., Wentworth, J. L., Heffron, C. L., & Meng, X.-J. (2015). Replacement of the hepatitis E virus ORF3 protein PxxP motif with heterologous late domain motifs affects virus release via interaction with TSG101. *Virology*, 486, 198–208.
- Koonin, E. V., Gorbalenya, A. E., Purdy, M. A., Rozanov, M. N., Reyes, G. R., & Bradley, D. W. (1992). Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: Delineation of an additional group of positive-strand RNA plant and animal viruses. *Proceedings of the National Academy of Sciences of the United States of America*, 89(17), 8259–8263.
- Lambert, C., Döring, T., & Prange, R. (2007). Hepatitis B virus maturation is sensitive to functional inhibition of ESCRT-III, Vps4, and gamma 2-adaptin. *Journal of Virology*, 81(17), 9050–9060.

- Lei, Q., Li, L., Zhang, S., Li, T., Zhang, X., Ding, X., & Qin, B. (2018). HEV ORF3 downregulates TLR7 to inhibit the generation of type I interferon via impairment of multiple signaling pathways. *Scientific Reports*, 8(1), 8585.
- Li, P., Liu, J., Li, Y., Su, J., Ma, Z., Bramer, W. M., ... Pan, Q. (2020). The global epidemiology of hepatitis E virus infection: A systematic review and meta-analysis. *Liver International*, 40(7), 1516–1528.
- Li, S.-W., Zhao, Q., Wu, T., Chen, S., Zhang, J., & Xia, N.-S. (2015). The development of a recombinant hepatitis E vaccine HEV 239. *Human Vaccines & Immunotherapeutics*, 11(4), 908–914.
- Li, T.-C., Takeda, N., Miyamura, T., Matsuura, Y., Wang, J. C. Y., Engvall, H., ... Cheng, R. H. (2005). Essential elements of the capsid protein for self-assembly into empty virus-like particles of hepatitis E virus. *Journal of Virology*, 79(20), 12999–13006.
- Manns, M. P., Wedemeyer, H., & Cornberg, M. (2006). Treating viral hepatitis C: Efficacy, side effects, and complications. *Gut*, 55(9), 1350–1359.
- Masciopinto, F., Giovani, C., Campagnoli, S., Galli-Stampino, L., Colombatto, P., Brunetto, M., ... Abrignani, S. (2004). Association of hepatitis C virus envelope proteins with exosomes. *European Journal of Immunology*, 34(10), 2834–2842.
- Medvedev, R., Ploen, D., Spengler, C., Elgner, F., Ren, H., Bunten, S., & Hildt, E. (2017). HCV-induced oxidative stress by inhibition of Nrf2 triggers autophagy and favors release of viral particles. *Free Radical Biology & Medicine*, 110, 300–315.
- Moin, S. M., Panteva, M., & Jameel, S. (2007). The hepatitis E virus Orf3 protein protects cells from mitochondrial depolarization and death. *The Journal of Biological Chemistry*, 282(29), 21124–21133.
- Montpellier, C., Wychowski, C., Sayed, I. M., Meunier, J.-C., Saliou, J.-M., Ankavay, M., ... Cocquerel, L. (2018). Hepatitis E virus lifecycle and identification of 3 forms of the ORF2 capsid protein. *Gastroenterology*, 154(1), 211–223.e8.
- Nagashima, S., Jirintai, S., Takahashi, M., Kobayashi, T., Tanggis, Nishizawa, T., ... Okamoto, H. (2014). Hepatitis E virus egress depends on the exosomal pathway, with secretory exosomes derived from multivesicular bodies. *The Journal of General Virology*, 95(Pt 10), 2166–2175.
- Nagashima, S., Takahashi, M., Jirintai, S., Tanaka, T., Nishizawa, T., Yasuda, J., & Okamoto, H. (2011). Tumour susceptibility gene 101 and the vacuolar protein sorting pathway are required for the release of hepatitis E virions. *The Journal of General Virology*, 92(Pt 12), 2838–2848.
- Nagashima, S., Takahashi, M., Jirintai, S., Tanggis, Kobayashi, T., Nishizawa, T., & Okamoto, H. (2014). The membrane on the surface of hepatitis E virus particles is derived from the intracellular membrane and contains trans-Golgi network protein 2. *Archives of Virology*, 159(5), 979–991.
- Nagashima, S., Takahashi, M., Jirintai, Tanaka, T., Yamada, K., Nishizawa, T., & Okamoto, H. (2011). A PSAP motif in the ORF3 protein of hepatitis E virus is necessary for virion release from infected cells. *The Journal of General Virology*, 92(Pt 2), 269–278.
- Nagashima, S., Takahashi, M., Kobayashi, T., Nishizawa, T., Nishiyama, T., Primadharsini, P. P., & Okamoto, H. (2017). Characterization of the quasi-enveloped hepatitis E virus particles released by the cellular exosomal pathway. *Journal of Virology*, 91(22), e00822-17.
- Nair, V. P., Anang, S., Subramani, C., Madhvi, A., Bakshi, K., Srivastava, A., ... Surjit, M. (2016). Endoplasmic reticulum stress induced synthesis of a novel viral factor mediates efficient replication of genotype-1 hepatitis E virus. *PLoS Pathogens*, 12(4), e1005521.
- Nanda, S. K., Panda, S. K., Durgapal, H., & Jameel, S. (1994). Detection of the negative strand of hepatitis E virus RNA in the livers of experimentally infected rhesus monkeys: Evidence for viral replication. *Journal of Medical Virology*, 42(3), 237–240.
- Owusu, I. A., Quaye, O., Passalacqua, K. D., & Wobus, C. E. (2021). Egress of non-enveloped enteric RNA viruses. *The Journal of General Virology*, 102(3), 1557.
- Parvez, M. K. (2017). The hepatitis E virus nonstructural polyprotein. *Future Microbiology*, 12, 915–924.
- Primadharsini, P. P., Nagashima, S., Takahashi, M., Kobayashi, T., Nishiyama, T., Nishizawa, T., ... Okamoto, H. (2020). Multivesicular body sorting and the exosomal pathway are required for the release of rat hepatitis E virus from infected cells. *Virus Research*, 278, 197868.
- Qi, Y., Zhang, F., Zhang, L., Harrison, T. J., Huang, W., Zhao, C., ... Wang, Y. (2015). Hepatitis E virus produced from cell culture has a lipid envelope. *PLoS One*, 10(7), e0132503.
- Rein, D. B., Stevens, G. A., Theaker, J., Wittenborn, J. S., & Wiersma, S. T. (2012). The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology*, 55(4), 988–997.
- Rodríguez-Furlan, C., Minina, E. A., & Hicks, G. R. (2019). Remove, recycle, degrade: Regulating plasma membrane protein accumulation. *Plant Cell*, 31(12), 2833–2854.
- Smith, D. B., Simmonds, P., Members Of The International Committee On The Taxonomy Of Viruses Study Group, Jameel, S., Emerson, S. U., Harrison, T. J., ... Purdy, M. A. (2014). Consensus proposals for classification of the family Hepeviridae. *The Journal of General Virology*, 95(Pt 10), 2223–2232.
- Sridhar, S., Teng, J. L. L., Chiu, T.-H., Lau, S. K. P., & Woo, P. C. Y. (2017). Hepatitis E virus genotypes and evolution: Emergence of camel hepatitis E variants. *International Journal of Molecular Sciences*, 18(4), 869.
- Sundquist, W. I., Schubert, H. L., Kelly, B. N., Hill, G. C., Holton, J. M., & Hill, C. P. (2004). Ubiquitin recognition by the human TSG101 protein. *Molecular Cell*, 13(6), 783–789.
- Surjit, M., Jameel, S., & Lal, S. K. (2004). The ORF2 protein of hepatitis E virus binds the 5' region of viral RNA. *Journal of Virology*, 78(1), 320–328.
- Surjit, M., Jameel, S., & Lal, S. K. (2007). Cytoplasmic localization of the ORF2 protein of hepatitis E virus is dependent on its ability to undergo retrotranslocation from the endoplasmic reticulum. *Journal of Virology*, 81(7), 3339–3345.
- Syed, S. F., Sun, Y., Du, T., Chen, Y., Liu, B., Wang, X., ... Zhao, Q. (2017). Evaluation of recombinant Chinese avian hepatitis E virus (CaHEV) ORF2 and ORF3 proteins for protection of chickens against CaHEV infection. *Vaccine*, 35(27), 3482–3489.
- Takahashi, M., Kobayashi, T., Tanggis, Jirintai, S., Mulyanto, Nagashima, S., ... Okamoto, H. (2016). Production of monoclonal antibodies against the ORF3 protein of rat hepatitis E virus (HEV) and demonstration of the incorporation of the ORF3 protein into enveloped rat HEV particles. *Archives of Virology*, 161(12), 3391–3404.
- Takahashi, M., Tanaka, T., Takahashi, H., Hoshino, Y., Nagashima, S., Jirintai, ... Okamoto, H. (2010). Hepatitis E virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the coexistence of HEV antibodies: Characterization of HEV virions in blood circulation. *Journal of Clinical Microbiology*, 48(4), 1112–1125.
- Takahashi, M., Yamada, K., Hoshino, Y., Takahashi, H., Ichiyama, K., Tanaka, T., & Okamoto, H. (2008). Monoclonal antibodies raised against the ORF3 protein of hepatitis E virus (HEV) can capture HEV particles in culture supernatant and serum but not those in feces. *Archives of Virology*, 153(9), 1703–1713.
- Tam, A. W., Smith, M. M., Guerra, M. E., Huang, C.-C., Bradley, D. W., Fry, K. E., & Reyes, G. R. (1991). Hepatitis E virus (HEV): Molecular cloning and sequencing of the full-length viral genome. *Virology*, 185(1), 120–131.
- Tanggis, Kobayashi, T., Takahashi, M., Jirintai, S., Nishizawa, T., Nagashima, S., ... Okamoto, H. (2018). An analysis of two open reading frames (ORF3 and ORF4) of rat hepatitis E virus genome using its infectious cDNA clones with mutations in ORF3 or ORF4. *Virus Research*, 249, 16–30.
- Tian, J., Shi, R., Xiao, P., Liu, T., She, R., Wu, Q., ... Soomro, M. H. (2019). Hepatitis E virus induces brain injury probably associated with mitochondrial apoptosis. *Frontiers in Cellular and Infection Microbiology*, 9, 433.

- Tyagi, S., Korkaya, H., Zafrullah, M., Jameel, S., & Lal, S. K. (2002). The phosphorylated form of the ORF3 protein of hepatitis E virus interacts with its non-glycosylated form of the major capsid protein, ORF2. *The Journal of Biological Chemistry*, 277(25), 22759–22767.
- Viswanathan, R. (2013). Infectious hepatitis in Delhi (1955-56): A critical study-epidemiology. 1957. *National Medical Journal of India*, 26(6), 362–377.
- Xing, L., Li, T.-C., Mayazaki, N., Simon, M. N., Wall, J. S., Moore, M., ... Cheng, R. H. (2010). Structure of hepatitis E virion-sized particle reveals an RNA-dependent viral assembly pathway. *The Journal of Biological Chemistry*, 285(43), 33175–33183.
- Xu, M., Behloul, N., Wen, J., Zhang, J., & Meng, J. (2016). Role of asparagine at position 562 in dimerization and immunogenicity of the hepatitis E virus capsid protein. *Infection, Genetics and Evolution*, 37, 99–107.
- Yamada, K., Takahashi, M., Hoshino, Y., Takahashi, H., Ichiyama, K., Nagashima, S., ... Okamoto, H. (2009). ORF3 protein of hepatitis E virus is essential for virion release from infected cells. *The Journal of General Virology*, 90(Pt 8), 1880–1891.
- Yamashita, T., Mori, Y., Miyazaki, N., Cheng, R. H., Yoshimura, M., Unno, H., ... Matsuura, Y. (2009). Biological and immunological characteristics of hepatitis E virus-like particles based on the crystal structure. *Proceedings of the National Academy of Sciences of the United States of America*, 106(31), 12986–12991.
- Yang, J.-F., Kao, Y.-H., Dai, C.-Y., Huang, J.-F., Hsieh, M.-Y., Lin, Z.-Y., ... Yu, M. L. (2010). Comparison of adverse effects related to pegylated interferon-based therapy for patients with chronic hepatitis B and chronic hepatitis C in Taiwan. *Hepatology International*, 4(4), 732–740.
- Yin, X., Ambardekar, C., Lu, Y., & Feng, Z. (2016). Distinct entry mechanisms for nonenveloped and quasi-enveloped hepatitis E viruses. *Journal of Virology*, 90(8), 4232–4242.
- Yin, X., Ying, D., Lhomme, S., Tang, Z., Walker, C. M., Xia, N., ... Feng, Z. (2018). Origin, antigenicity, and function of a secreted form of ORF2 in hepatitis E virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, 115(18), 4773–4778.
- Zafrullah, M., Ozdener, M. H., Panda, S. K., & Jameel, S. (1997). The ORF3 protein of hepatitis E virus is a phosphoprotein that associates with the cytoskeleton. *Journal of Virology*, 71(12), 9045–9053.

How to cite this article: Glitscher, M., & Hildt, E. (2021).

Hepatitis E virus egress and beyond – the manifold roles of the viral ORF3 protein. *Cellular Microbiology*, e13379. <https://doi.org/10.1111/cmi.13379>