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Peek-a-boo: membrane hijacking and the pathogenesis of viral hepatitis

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Historically, animal viruses have been classified on the basis of the presence or absence of an envelope – an external lipid bilayer membrane typically carrying one or more viral glycoproteins. However, growing evidence indicates that some 'non-enveloped' viruses circulate in the blood of infected individuals enveloped in host-derived membranes that provide protection from neutralizing antibodies. In this opinion article, we discuss this novel strategy for virus survival and consider how it contributes to the pathogenesis of acute viral hepatitis. The acquisition of an envelope by non-enveloped viruses profoundly influences their interaction with the host at both the cellular and system level and challenges how we think about vaccine protection against these infections.

To have and have not: viruses and envelopes

Virologists, like all scientists, are drawn to classifying the things they study. As of 2012, the International Committee on the Taxonomy of Viruses (ICTV) had agreed on the existence of 2618 species of viruses, spread across 420 genera, 96 families, and seven orders [1]. The classification of viruses increasingly rests on evolutionary relationships inferred from phylogenetic analyses of virus sequences, but for many decades viruses were classified based on a small number of key physical attributes: size, shape, type of nucleic acid, and the presence or absence of an envelope as reflected in susceptibility to ether or bile-salt inactivation. Old habits die slowly and even today most virologists maintain a dichotomous view of viruses, placing them into one of two bins: those with and those without an envelope – that is, an outer lipid bilayer derived from cellular membranes in which host membrane proteins have been largely replaced by viral glycoproteins (peplomers). The envelope is essential for those viruses that possess one, because the peplomers embedded within it are typically key determinants of cell tropism and entry. By contrast, the absence of an envelope can confer specific advantages, including greater environmental stability. Such attributes have substantial impact on how viruses are transmitted and how they are seen by the host immune system.

Life is never simple, however, and there are now examples of viruses that exist outside the cell in two equally infectious guises: one with and one without an envelope. How and why do these viruses do it? Also, what does this mean for our ability to prevent these infections with a vaccine? We focus here on two such viruses, hepatitis A virus (HAV) and hepatitis E virus (HEV), both considered by virologists for many years to be non-enveloped viruses but recently recognized to have a more complicated *modus operandi*. HAV, a member of the non-enveloped family *Picornaviridae*, has evolved a unique strategy by which it hijacks cellular membranes to exit cells fully cloaked in a lipid membrane [2]. HEV, which may have evolved from an ancestral enveloped virus [3], appears to have acquired the ability to shed its envelope and the liabilities it poses for transmission through the environment [4]. Their unusual lifestyle allows these viruses to evade neutralizing antibodies and facilitates their spread within infected hosts while maximizing opportunities for inter-host transmission.

HAV and HEV – viruses with dual personalities

HAV and HEV are phylogenetically unrelated, small RNA viruses, each with a single-stranded, positive-sense genome (Figure 1). Both infect the liver and cause acute inflammatory hepatitis [5,6]. Remarkably, they circulate in the blood during acute infection in a membrane-enveloped form, but are shed in feces as non-enveloped viruses [2,4]. The two

Glossary

Exosomes: small, 50–120-nm, single-membrane vesicles released from numerous cell types via fusion of MVBs to the plasma membrane that carry various biologically active protein and RNA cargos and that are increasingly recognized to play important roles in intercellular communication.
Multivesicular bodies (MVBs): specialized late endosomes that contain multiple internal vesicles formed by inward budding of the endosomal membrane in a process mediated by ESCRT complexes. MVBs may traffic to and fuse with lysosomes or move to the plasma membrane, where fusion of their outer membrane results in release of their internal vesicles as exosomes.
Ockham's razor: a philosophical and scientific tenet attributed to a 14th century friar that states in effect that the simplest explanation for a particular phenomenon is to be preferred over more complicated explanations with unnecessary assumptions.

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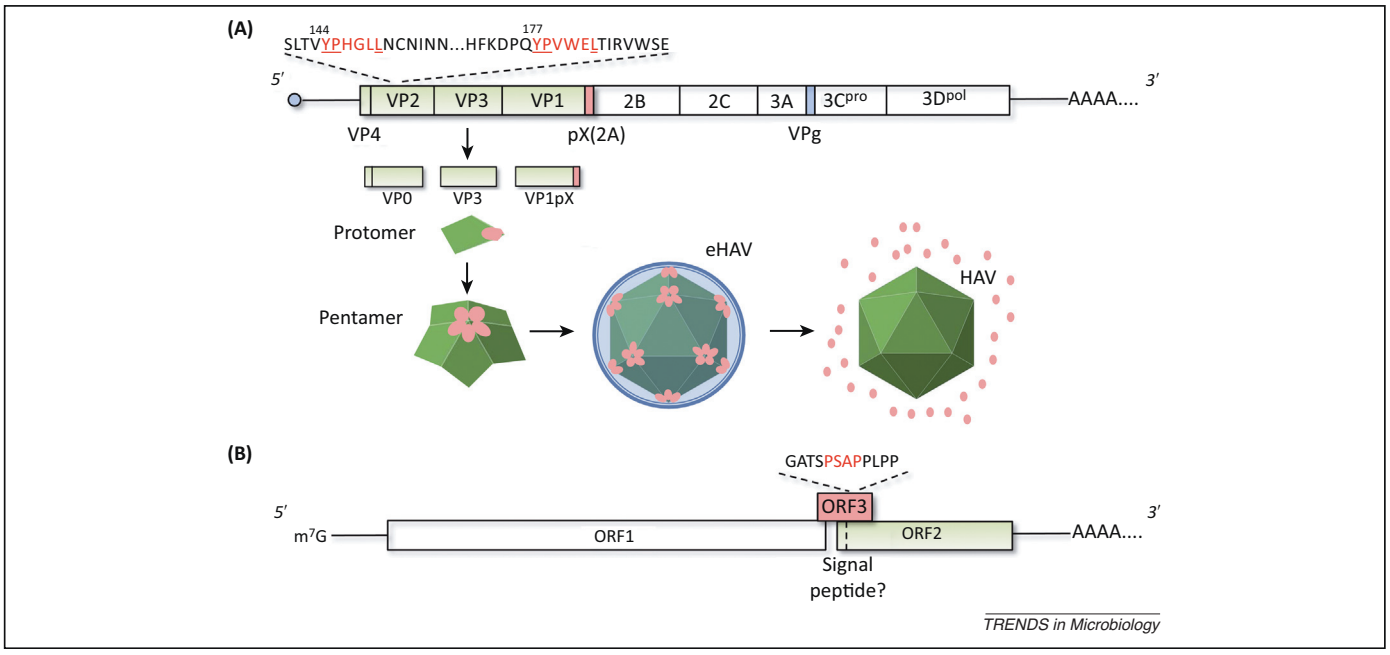

Figure 1. Organization of the 3′ polyadenylated, positive-strand RNA genomes of hepatitis A virus (HAV) and hepatitis E virus (HEV). (**A**) The HAV genome contains one large open reading frame (ORF) (box) that encodes a giant polyprotein that is processed into nine or more mature proteins. The three major capsid proteins, VP0, VP3, and VP1 (shaded in green), are processed from the polyprotein by the viral protease $3C^{pro}$ and form protomers that then assemble into pentamers in a process dependent on pX, an 8-kDa carboxy-terminal extension of VP1 (pink). Pentamers subsequently assemble into an icosahedral capsid that packages the viral genome and is released from cells wrapped in cellular membranes (eHAV). pX is stable within the eHAV particle, but is degraded following dissolution of the membrane. Tandem YPX_3L motifs (red font) in VP2 are likely to interact with the endosomal sorting complex required for transport (ESCRT)-associated protein Alix and, together with pX, direct the membrane envelopment of the capsid. (**B**) The HEV genome contains three ORFs, the largest and most 5′ of which (ORF1) encodes nonstructural proteins that direct its replication. The most 3′, ORF2, encodes the capsid protein that appears to have an amino-terminal signal sequence. ORF3 is required for the release of virus from cells enveloped in membranes and contains a PSAP late domain mediating interactions with the ESCRT-associated protein TSG101. The HEV genome terminates at the 5′ end with a m^7G cap, whereas the HAV genome is covalently linked to a small viral protein (VPg) and is translated under the control of an internal ribosome entry site in its 5′ untranslated RNA segment.

types of particle, enveloped and non-enveloped, appear to be equally infectious, but in both cases the enveloped viruses are highly resistant to neutralizing antibodies whereas the non-enveloped particles are not [2,4]. This remarkable convergence of phenotypes is likely to relate in part to the unique setting of the liver within which these viruses replicate and for which the biliary tract provides an efficient conduit to the outside environment (Figure 2A).

HAV is an ancient human pathogen and a common cause of enterically transmitted acute viral hepatitis [5]. Its genome organization and phylogeny classify it squarely as the sole member of a distinct genus, the *Hepatoviruses*, within the family *Picornaviridae* [7]. As with all picornaviruses, the genome is released from the HAV capsid during the process of viral entry, with an internal ribosome entry site in the 5′ untranslated RNA segment then initiating the translation of a giant polyprotein that is subsequently processed into nine mature proteins as well as several functional processing intermediates [8] (Figure 1A). The major capsid proteins, VP0, VP3, and VP1pX, assemble in the cytoplasm as pentamers, with 12 pentamers subsequently forming a viral capsid. pX is an 8-kDa carboxy-terminal extension of VP1 that is cleaved from it by an unknown cellular protease late in maturation of the virion [9–11]. HAV replication is slow and generally non-cytolytic in cell culture. Infectious virus is released into supernatant fluids [12], but how this occurs in the absence of cell lysis was unexplained for many years.

Recent work reveals that the virus released into supernatant fluids comprises two populations of infectious particles with distinctly different morphology, buoyant density, and resistance to neutralizing antibodies [2]. Under the electron microscope, virus banding at approximately 1.22 g/cm^3 in isopycnic iodixanol gradients has the size and shape of HAV particles first identified by Feinstone *et al.* in 1973 in human feces [13]. However, a greater proportion of the virus bands at a much lighter density, approximately 1.08 g/cm^3, and comprises similar-appearing capsids enveloped in a single, amorphous lipid bilayer [2]. Most of these vesicle-like structures contain one or two capsids but a few contain as many as four, suggesting that the membrane is acquired after capsid assembly. Surprisingly, viruses in the light and dense fractions appear to have equivalent infectivity (infectious focus-forming units/genome copy). Consistent with being fully enveloped in membranes, the light particles are resistant to neutralizing antibodies [2]. Their infectivity is virtually eliminated by extraction with chloroform, because the membrane-associated virus partitions into the interface and is lost from the aqueous phase. Given that HAV has been classified for many years among the picornaviruses, a large and diverse family of non-enveloped viruses, it was surprising to find that most virions released into the supernatant fluids of infected hepatoma cell cultures were enveloped in this fashion. Even more surprising, only the enveloped form of the virus (eHAV) was found in the blood of patients with acute hepatitis A [2]. By contrast, virus shed in feces lacks an envelope, similar to any standard picornavirus.

Less is known about HEV. It causes acute enterically transmitted hepatitis, similar to HAV, and has been