Chapter 15
Beyond Channel Activity: Protein-Protein Interactions Involving Viroporins

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The Viroporins and Channel Activity Inhibition

The field of viroporin research has its origins in the observation that virus-infected cells show increased membrane permeability (Carrasco 1995). More than two decades later, viroporins have been confirmed in several viral families, e.g., *Orthomyxoviridae* (AM2, PB1-F2, BM2), *Flaviviridae* (p7), *Coronaviridae* (E, 3a, 4a), *Paramyxoviridae* (SH), *Picornaviridae* (2B/2 BC, 3A), *Togaviridae* (6 K), *Retroviridae* (Vpr, Vpu, p13), *Reoviridae* (NSP4 and p10), *Polyomaviridae* (agnoprotein, VP2-VP4), *Papillomaviridae* (E5), or *Rhabdoviridae* (α1). Currently, detailed structural information is limited to only a handful of viroporins (*vide infra*), although these constitute useful templates for the probably hundreds of other unknown viroporins yet to be discovered in reservoir hosts (Anthony et al. 2013).

In most cases, viral attenuation is not only achieved by deletion of the viroporin gene but also simply when their channel activity is suppressed. Indeed, various specific pathogenic roles of viroporin channel activity have been discovered, and attempts have been made to modulate this channel activity, especially that of influenza A virus M2 (IAV M2, or AM2) protein, the first discovered and the best characterized viroporin. In general, however, the road to rational design and discovery of viroporin small-molecule inhibitors has not been successful [see To et al. (2016) for a recent review]. In fact, amantadine and rimantadine are at present the only licensed antiviral drugs that target a viroporin, i.e., IAV M2. However, most circulating strains of IAV are Amtresistant (Deyde et al. 2007; Hayden and De Jong 2011), and neither drug is currently being used in humans.

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Protein-Protein Interactions (PPIs) Involving Viroporins

Viroporins are involved in many protein-protein interactions (PPIs) that may be also susceptible to therapeutic intervention [see recent reviews (Fischer et al. 2014; Nieva and Carrasco 2015)]. Both intraviral and virus-host interactions, in the form of a myriad of perturbations, can provide important insights into the mechanisms involved in the viral infectious cycle. Also, understanding these PPI networks may aid the design of new antivirals. These strategies depend on both detailed structural and mechanistic information and on the availability of therapeutically relevant targets. In this sense, the initial focused approach to identify viroporin binders is being complemented with genome-wide interactome studies adapted to viral infections using high-throughput technologies, providing a dramatic boost in the search for possible PPIs [see de Chassey et al. (2014) for a recent review].

Useful methods to obtain leads for PPIs involving viroporins include yeast two-hybrid (Y2H) screens, e.g., the genome-wide virus-host PPI screen of HCV was performed almost 10 years ago using a construction of a viral ORFeome and Y2H technology (De Chassey et al. 2008), identifying hundreds of PPIs involving viral and host proteins, 13 of which involving p7 and proteins expressed in the liver. Other Y2H screens have included IAV M2 virus-virus and virus-host interactions (Shapira et al. 2009), or intraviral PPIs in the coronavirus responsible for severe acute respiratory syndrome (SARS-CoV) (von Brunn et al. 2007). However, this method does not measure interactions between proteins in the context of the infected cell, is biased against membrane proteins, and cannot study protein complexes that are weakly or transiently associated. The bias against membrane proteins can be compensated using the split-ubiquitin-based yeast two-hybrid screen, e.g., in a screen to search for binders of the small hydrophobic (SH) protein of the respiratory syncytial virus (RSV) (Li et al. 2015).

More suitable methods detect interactions in the context of the infected cell, using a combination of affinity purification with mass spectrometry, e.g., Wang et al. (2017), although the method has also low sensitivity. Other methods are based on microarrays of deposited purified proteins (Zhu et al. 2001) and protein complementation assay (PCA) (Tarassov et al. 2008). Lastly, an approach that combines on-chip in vitro protein synthesis with an in situ microfluidic affinity assay can detect even weak or transient interactions (Gerber et al. 2009), although host-virus PPIs using this method so far has only been tested for M protein in RSV (Kipper et al. 2015).

From these studies, it is apparent that viroporins, and viral proteins in general, tend to show preference for key host proteins that have a high number of direct interacting partners. Also, interactions may be simultaneous with many cellular proteins, making use of intrinsically disordered protein regions enriched for short linear motifs, e.g., PDZ-binding motifs (Hagai et al. 2014; Meyniel-Schicklin et al. 2012), to compensate for their small proteomes.

The Influenza A Virus Matrix Protein 2 (IAV M2 or AM2)

Influenza viruses belong to the *Orthomyxoviridae* family of segmented, negative-sense, enveloped RNA viruses. The seasonal flu caused by the influenza A virus (IAV) is known for causing pandemics with high mortality rates (Hay et al. 2001; Neumann et al. 2009), although its close relative influenza B accounts for half of the influenza disease in recent years (www.cdc.gov). Generally, influenza virions are spherical in shape ranging from 80 to 120 nm in diameter, although filamentous forms may also occur (Lamb and Choppin 1983). The viral envelope contains three transmembrane proteins, hemagglutinin (HA), neuraminidase (NA), and matrix protein 2 (M2), on the outside and a layer of matrix protein (M1) just underneath the membrane that contains cholesterol-enriched lipid rafts. M1 forms an internal coat that encloses the viral ribonucleoproteins (vRNPs), i.e., the negative-strand viral RNA (vRNA) and nucleoprotein (NP), with small amounts of the nuclear export protein (NEP) and three polymerase (3P) proteins (PA, PB1, and PB2) that form the viral RNA polymerase complex (Fields et al. 2013).

M2 Viroporin

The viroporin M2 in IAV is a homotetrameric channel (Sakaguchi et al. 1997). Each M2 monomer is a 97-amino acid protein comprising an N-terminal ectodomain (24 aa), an α-helical transmembrane domain (TMD, 19 aa), and a highly conserved cytoplasmic tail (CT) domain (54 aa) that is a hotspot for interactions with both viral and host proteins during the IAV life cycle. The latter may therefore constitute an attractive drug target for the development of IAV antivirals. M2 has a pH-activated proton channel activity which is required to complete the uncoating process during virus entry. Upon virus internalization via endocytosis, M2 selectively conducts protons from acidified endosomes into the viral interior. This acidification of virion triggers the dissociation of the M1 protein from the vRNP complex, thereby enabling the transport of vRNPs into the nucleus for replication of viral genetic material (Helenius 1992). For some IAV subtypes, the M2 proton channel raises the pH of the trans-Golgi network (TGN) to protect the viral HA from premature low-pH conformational change during its transport to the cell surface (Takeuchi and Lamb 1994). The channel activity of IAV M2 has been found to be sufficient for the activation of the NLRP3 inflammasome in influenza-infected cells (Ichinohe et al. 2010). Presently, there are more than ten structures of both wild-type and drug-resistant mutant M2 channels in the Protein Data Bank [see review in Gu et al. (2013)].

M1-M2 Interaction

Early works suggested a role for IAV M1-M2 interaction in virus budding and control of virion morphology (filamentous versus spherical). Interaction between M1 with the M2 cytoplasmic tail was first suggested from the analysis of escape mutants (Zebedee and Lamb 1989). Further work revealed a physical interaction between M1 and the M2 cytoplasmic tail at the site of virus budding, to facilitate virus assembly by promoting the recruitment and packaging of viral proteins and viral genome (McCown and Pekosz 2006; Chen et al. 2008). The cytoplasmic tail of M2 contains an amphipathic α-helix (residues 45–62) (Schnell and Chou 2008) that can modulate membrane curvature in a cholesterol-dependent manner. This feature of M2 has been proposed to be implicated in (i) modification of local membrane curvature during virus budding to provide a stabilized scaffold for M1 polymerization and virus filament formation and (ii) alteration of membrane curvature at the neck of budding virions to facilitate membrane scission and virion release (Rossman and Lamb 2011).

Host Interactions

In addition to intraviral interactions, a number of interactions of M2 with host proteins have been described to modulate autophagy, membrane trafficking, host defense, and virus budding. For example, IAV M2 has been reported to arrest autophagy (Gannagé et al. 2009; Beale et al. 2014), a cellular degradation pathway mediated by autophagosomes which delivers cytoplasmic materials to the lysosome that is regulated by autophagy-related genes (Atg). This process involves (i) target engulfment by an isolation crescent membrane (phagophore) to form the autophagosome and (ii) autophagosome-lysosome fusion and degradation of the intra-autophagosomal contents (Fig. 15.1a). IAV subverts this machinery by blocking this fusion, resulting in increased apoptosis of IAV-infected cells. In IAV-infected A549 human lung epithelial cells, M2 coimmunoprecipitates with Atg6/Beclin-1 through interaction with M2 residues 1–60 (Gannagé et al. 2009). Atg6/Beclin1 is part of a complex that regulates autophagosome generation and degradation and is a common target of other viruses for the subversion of autophagy, e.g., herpesviruses and the human immunodeficiency virus (HIV) [reviewed in Münz (2011)].

The C-terminal tail of M2 has also been implicated in the binding to LC3, a protein that normally localizes to autophagosomal membranes (Sou et al. 2006) to recruit autophagy receptors carrying substrates destined for autophagic degradation. These receptors typically contain an LC3-interacting region (LIR), with consensus LIR motif W/FxxI/L/V. In IAV-infected cells, the localization of LC3 changes from the cytoplasm and autophagosomal membranes to the plasma membrane (Beale et al. 2014), a change mediated by a putative LIR motif (residues 91–94, FVSI)

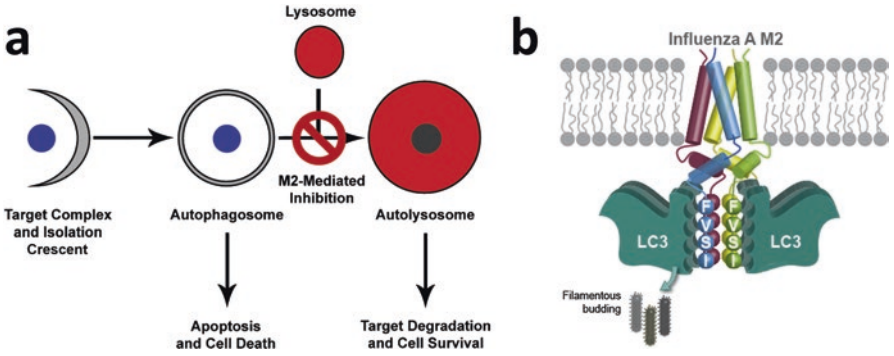

Fig. 15.1 IAV M2 may subvert autophagy by (a) interaction with Atg6/Beclin-1 to block autophagosome maturation, triggering apoptosis [adapted from Rossman and Lamb (2009)] and (b) interaction with LC3 through an M2 LIR motif (FVSI) that hijacks LC3 to the plasma membrane [adapted from Beale et al. (2014)]

present in the cytoplasmic tail of M2 (Fig. 15.1b). Binding of LC3 to the LIR motif of M2 has been confirmed by LUMIER binding assays (Barrios-Rodiles et al. 2005) and GFP pull-down experiments.

The M2-LC3 interaction is also a factor in the budding of IAV, which, depending on the viral strain and host cell type, can produce either spheres or filaments (Bourmakina and García-Sastre 2003), with the latter requiring extensive membrane resources. Cells infected with a filamentous budding IAV strain carrying mutations in the M2 LIR motif that abolished M2-LC3 interactions produced fewer filaments than cells infected with wild-type IAV, suggesting that hijacking of LC3 by M2 may assist in the delivery of LC3-conjugated membranes to the cell surface to facilitate IAV budding (Beale et al. 2014).

The cytoplasmic domain of IAV M2 has been reported to bind caveolin-1 (Cav-1) (Zou et al. 2009), a raft-residing cholesterol-binding protein implicated in the life cycle of viruses that buds from lipid rafts, such as HIV, RSV, and rotavirus. Most Cav-1-associated proteins contain an aromatic-rich caveolin-binding motif (CBM), a consensus sequence of aromatic residues separated by a specific spacing (Couet et al. 1997). The M2-Cav-1 interaction has been confirmed by pull-down and coimmunoprecipitation assays, with the putative CBM in M2 proposed to reside in the cytoplasmic, juxtamembrane region of the M2 tail (Sun et al. 2010). This interaction suggests that Cav-1 may modulate virus budding, possibly through the trafficking of M2 to the plasma membrane.

A yeast two-hybrid screening effort identified the transport protein particle complex 6A (TRAPPC6A) and also its N-internal deleted isoform, TRAPPC6AΔ, as binders to the last six amino acids at the C-terminal end of IAV M2, with highly conserved Leu96 located at the extremity of M2 being indispensable in mediating the interaction (Zhu et al. 2017). TRAPP complexes are multi-subunit tethering

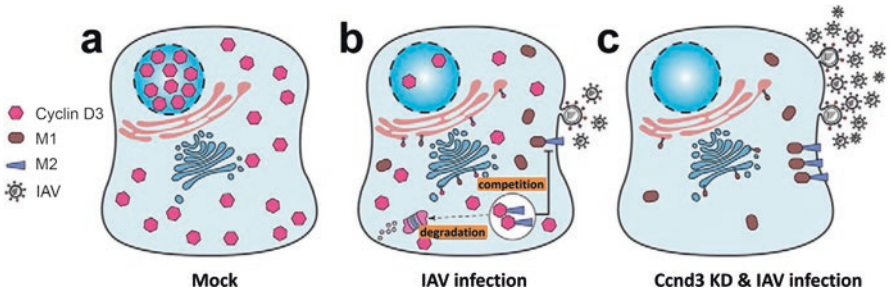

Fig. 15.2 Proposed activity of cyclin D3 in IAV budding. (**a**) In normal cells, cyclin D3 is localized to the nucleus where it regulates cell cycle; (**b**) during IAV infection, cyclin D3 interacts with viral M2 to disrupt M1-M2 binding; (**c**) in the absence of cyclin D3, more infectious progeny virus particles are released [adapted from Fan et al. (2017)]

complexes involved in intracellular membrane trafficking pathways, and TRAPPC6AΔ may be a regulator of M2 transport to the cell surface.

A yeast two-hybrid assay combined with mutagenesis identified the binding of both AM2 and BM2 to the C-terminal domain 1 (CTD1) of Hsp40 (Guan et al. 2010), a molecular chaperone that can regulate the critical PKR signaling pathway against viral infection. Hsp40 associates with the PKR inhibitor, $P58^{IPK}$. Binding studies suggest that M2 also binds to $P58^{IPK}$, possibly forming a stable complex with both Hsp40 and $P58^{IPK}$ (Guan et al. 2010), which would enhance PKR autophosphorylation and activation to inhibit host protein synthesis.

Results from another yeast two-hybrid screen using the M2 cytoplasmic tail as a bait have identified the human cell cycle regulator cyclin D3 as a binder, and their interaction has been confirmed in infected cells by immunoprecipitation assays (Fan et al. 2017). Using siRNA-mediated knockdown of cyclin D3 expression, the study proposed that cyclin D3 is a negative modulator of IAV infection, competing with M1 for binding to M2 in transfected cells and therefore disrupting the important M1-M2 interaction in the context of an IAV infection. On the other hand, IAV may antagonize cyclin D3 activity by (i) relocating cyclin D3 from the nucleus (Fig. 15.2a) to the cytosol (Fig. 15.2b) to facilitate virus replication by promoting cell cycle arrest and (ii) targeting cyclin D3 for cytosolic proteasomal degradation to prevent its interference with M1-M2 binding (Fig. 15.2b). Cyclin D3 has been also reported to be a target of SARS-CoV viroporin 3a (Yuan et al. 2007)

A recent study has identified M2 as a putative viral antagonist of BST-2 (bone marrow stromal cell antigen 2, also known as tetherin, CD317) (Hu et al. 2017), a protein that may restrict the release of infectious IAV (Mangeat et al. 2012). BST-2 can inhibit the release of a wide range of enveloped viruses by tethering budding virions to the cell surface [reviewed in le Tortorec et al. (2011)], e.g., in HIV-1, in that case antagonized by its viroporin Vpu (Neil et al. 2008). However, the role of BST-2 in limiting IAV release has been disputed (Watanabe et al. 2011), perhaps due to IAV strain-specific susceptibility to BST-2 restriction. This interaction was confirmed by orthogonal assays, and using chimeric and truncated M2, the regions

involved in BST-2 downregulation were proposed to be within the M2 extracellular and TMDs.

Another report, using a Y2H screening with M2 cytoplasmic tail as bait, discovered the binding to annexin A6 (AnxA6) (Ma et al. 2012), a Ca^{2+}-regulated membrane-binding protein that controls intracellular cholesterol homeostasis, and regulates membrane fusion and vesicle formation in endocytic and exocytic pathways (Raynal and Pollard 1994). This interaction has been verified by coimmunoprecipitation assays and colocalization studies in infected cells (Ma et al. 2012). Modulation of AnxA6 expression led to corresponding variations in production of infectious IAV, suggesting that AnxA6 is a negative modulator of IAV infection (Ma et al. 2012), as it may impair IAV budding and the release of progeny virus.

A Y2H screen also identified Na^+/K^+ ATPase β1 subunit (ATP1B1) as binder to the M2 cytoplasmic domain (Mi et al. 2010). SARS-CoV E viroporin and the human papillomavirus E5 viroporin have also been found to bind the host Na^+/K^+ ATPase α1 subunit (Nieto-Torres et al. 2011) and vacuolar H^+ ATPase (Andresson et al. 1995), respectively.

PB1-F2 Viroporin

Another protein in IAV, PB1-F2 (Chen et al. 2001), has the hallmarks of a viroporin: it is ~90 residues long, it forms oligomers, and it has been shown to form a nonselective ion channel in planar lipid bilayers and microsomes (Henkel et al. 2010). PB1-F2 is known to induce apoptosis in host immune cells (Chen et al. 2001) via interaction with two mitochondrial proteins, ANT3 and VDAC1, resulting in the loss of mitochondria membrane potential (Varga et al. 2012), although its localization and pro-apoptotic behavior is strain and cell type specific (Varga and Palese 2011). In addition, PB1-F2 from pathogenic strains of IAV can be incorporated into the phagolysosomal compartment to activate the NLRP3 inflammasome, resulting in IL-1β secretion and causing severe pathophysiology (McAuley et al. 2013). Also, binding of PB1-F2 of PR8 to MAVS, a RIG-I-like receptor (RLR) signaling adaptor anchoring to mitochondria, led to antagonism activity on interferon production (Varga et al. 2012). Despite these data, the precise role of PB1-F2 in modulation of IAV-induced immunopathogenesis is still unknown.

IAV Protein Interactome

Many more IAV host-virus PPIs have been detected recently using affinity purification coupled with mass spectrometry in the context of infected cells. For example, the interactome of 11 viral proteins of influenza PR8 IAV and another 3 strains was analyzed (Wang et al. 2017), confirming that M2 protein is one of the major nodes

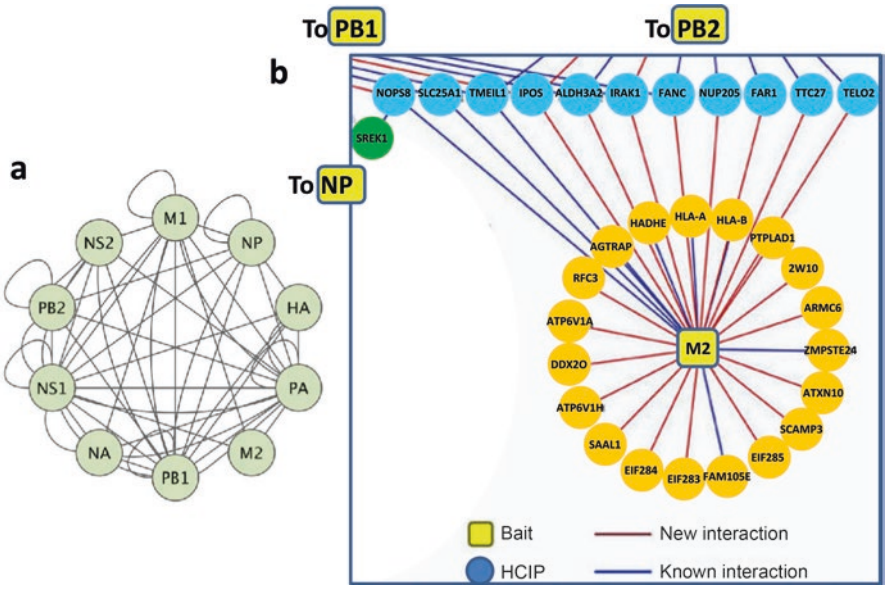

Fig. 15.3 Maps of the IAV intraviral and virus-host protein interactome. (**a**) Result from a Y2H study to identify direct binary contacts among the ten major viral proteins of the PR8 strain (Shapira et al. 2009). This study also detected nine interactions between IAV M2 and host proteins, among them RNA-binding proteins, transcription factors, or proteins involved in signaling pathways, or to detect intraviral PPIs; (**b**) close-up of high-confidence candidate-interacting proteins (HCIPs) associated with multiple IAV strains when M2 was used as bait. Indicated are links to other viral proteins (NP, PB1, and PB2) [adapted from Wang et al. (2017)]

connecting host proteins with roles in immunity and regulation of viral infection. Almost 100 interactions of host with M2 protein were detected, and ~30 were common to at least three of the strains (Fig. 15.3).

The Hepatitis C Virus p7 Protein (HCV-p7)

The hepatitis C virus (HCV) is an enveloped positive-strand RNA virus member of the *Flaviviridae* family (genus *Hepacivirus*) that has chronically infected 170 million people worldwide, causing human liver disease. Hepatitis C is divided into six genotypes, with genotype 1 being the most common and most difficult to treat. Treatment against HCV infection involves drugs targeting both viral and host proteins, e.g., drugs that target NS3/4A protease, the NS5A protein, or the NS5B RNA-dependent RNA polymerase (RdRP). However, the rapid turnover of HCV replication (Neumann et al. 1998) and the error-prone activity of the HCV RNA polymerase lead to a rapid formation of "quasi-species" and therefore resistance to antivirals.

HCV encodes a single polyprotein of ~3000 amino acids that is cleaved by cellular and viral proteases into ten different proteins: three structural proteins (core, E1, and E2) and seven nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). The structural proteins Core (C), E1, and E2 are located in the N-terminal region and form the viral particle (Moradpour and Penin 2013), whereas NS3 to NS5B are involved in the replication of the viral genome. p7 and NS2 are dispensable for RNA replication but are critical for virion morphogenesis, which requires both structural and nonstructural proteins (Appel et al. 2008; Steinmann et al. 2007), although the latter are not packaged in viral particles.

HCV replication takes place in double-membrane vesicles (DMVs), while viral assembly sites (AS) have been suggested to be specialized detergent-resistant membranes (DRMs) in the ER or in mitochondria-associated ER membranes rich in cholesterol and sphingolipids (Shanmugam et al. 2015). The core protein concentrates at cytosolic lipid droplets (cLDs) close to the ER-located assembly site and is eventually linked to the vRNA replication site in specialized ER-derived structures.

During HCV assembly, one of the first steps is the interaction of cLD-bound core protein and NS5A (Appel et al. 2008). NS2, probably in complex with p7, interacts with the NS3-4A enzyme, and this retrieves the viral core protein from cLDs into the nascent virus particles [reviewed in Lindenbach and Rice (2013)]. Virus particles transit through the secretory pathway, where they are protected from exposure to low pH by p7, which neutralizes intracellular compartments (Wozniak et al. 2010). More recently, a genetic interaction has been observed between p7 and NS5B proteins, which were found to cooperate to promote virion infectivity by decreasing sphingomyelin content in the virion (Aligeti et al. 2015).

p7 Viroporin The viroporin p7 is produced when E2-p7-NS2 is cleaved by a signal peptidase at the ER (Lin et al. 1994; Mizushima et al. 1994). p7 is a 63-residue-long protein that has two α-helical TMDs and is found mainly at the ER membrane. As mentioned above, p7 is essential for virus particle assembly and release (Steinmann et al. 2007) and for productive HCV propagation in vivo (Sakai et al. 2003), but not necessary for RNA replication. The bovine viral diarrhea virus (BVDV) and the hepacivirus GB virus B (GBV-B), HCV's closest relatives, also have a p7 protein crucial for virus replication.

p7 has channel activity with low cation selectivity (Griffin et al. 2003; Ouyang et al. 2013). p7 has been reported to permeabilize membranes to protons, preventing the acidification of intracellular vesicles (Wozniak et al. 2010), an activity that has been confirmed in vitro using a liposome-based assay (Gan et al. 2014).

The structural model for p7 is that of an α-helical hairpin with two α-helical TMDs kinked in the middle (Cook et al. 2010), or a sequence divided into three helical segments (Ouyang et al. 2013) where the N-terminal half of the polypeptide would face the lumen of the channel and the C-terminal helix, p7(27–63), faces the lipid environment. The channel is formed by either six or seven monomers (Luik et al. 2009; Montserret et al. 2010).

p7-NS2 Interaction

Early genetic analyses suggested that p7 interacts with NS2, a polytopic membrane protein containing three N-terminal TMDs that is essential in the assembly process of the HCV particle (Jirasko et al. 2008). Mutation of residues in one protein to induce the emergence of complementary mutations in the other was used to identify the interaction network of NS2 protein with p7, E1 and E2, and NS3 proteins (Jirasko et al. 2010). Similar conclusions were reached in studies involving chimeric constructs with different genotypes (Pietschmann et al. 2006) which showed that virus release was most efficient when the N-terminal TMD of NS2 was from the same isolate as the core-to-p7 region. In a similar study, adaptive mutations in E1, p7, NS2, and NS3 were detected that were essential for virus assembly and/or release, again suggesting genetic interactions between these proteins (Yi et al. 2007).

Physical interaction between p7 and NS2 was observed during pull-down assays and mutagenesis (Ma et al. 2011), which suggested that p7 may regulate NS2-mediated complexes that are crucial for production of infectious HCV particles. Coimmunoprecipitation and FRET assays also supported a physical interaction between p7 and NS2 (Popescu et al. 2011), suggesting a complex between p7, NS2, and E2 mediated by transmembrane interactions. These interactions were proposed to be required to localize NS proteins and the core-containing cLDs to sites of virus assembly. Overall, these studies demonstrated that NS2, together with p7 protein, plays a central organizing role in HCV particle assembly by bringing together viral structural and nonstructural proteins. Although the exact mechanism linking nucleocapsid assembly with envelope acquisition is unknown, p7 and NS2 have been proposed to play a critical role in the migration of core protein and E1-E2 heterodimers to the virion assembly site (Vieyres et al. 2014).

Another role, in immune evasion, has been identified recently for p7 (Qi et al. 2017). Indeed, HCV acts against the host immune system by downregulating interferon (IFN) production, blocking IFN signaling transduction, and impairing IFN-stimulated gene (ISG) expression. But even when ISGs are expressed, most ISGs have been reported to be ineffective when overexpressed in virus-infected cells due to unknown mechanisms (Schoggins et al. 2011). By constructing a library of mutant HCVs with a 15-nt insertion, p7 was identified as an immune evasion protein that suppresses the antiviral IFN function, forming a complex with the host interferon-inducible protein 6–16 (IFI6–16) that has been verified by coimmunoprecipitation. It was proposed that while IFI6–16 acts to stabilize the mitochondrial membrane potential, p7 counteracts by depolarizing the mitochondrial potential, likely through its ion channel activity. Overall, the findings suggest that p7 antagonizes the antiviral responses of IFN by inhibiting the antiviral function of IFI6–16.

HCV Protein Interactome

A proteome-wide virus-host PPI screen of HCV was performed almost 10 years ago using a construction of a viral ORFeome and Y2H technology (De Chassey et al. 2008). In that study, 314 PPIs were identified involving viral and host proteins, 13 of which involving p7 and proteins expressed in the liver (Fig. 15.4), although these were not confirmed by orthogonal methods. A latter study combined mass spectrometry and functional genomics (Germain et al. 2014), but p7 was not included in the screen.

The interaction network between the ten HCV proteins has been investigated using a flow-cytometry-based FRET assay in living cells. In this study, p7 was found to bind NS2, Core, E1, and E2 (Hagen et al. 2014) (Fig. 15.5a). In 2016, a computational coevolution analysis of HCV attempted to reconstruct the PPI network of the HCV at the residue resolution (Champeimont et al. 2016). Coevolving residues were identified to predict PPIs for further experimental identification of HCV protein complexes (Fig. 15.5b). One of these interactions was p7-NS2 (see Fig. 15.5c).

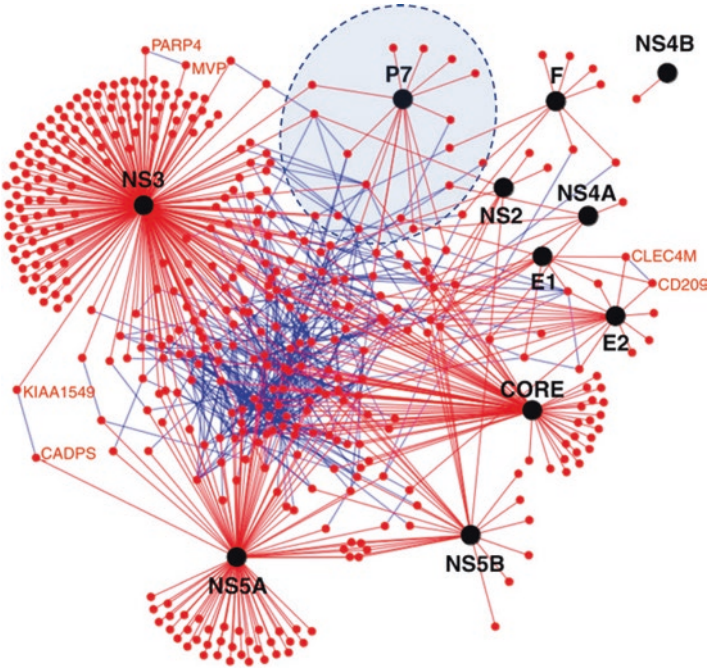

Fig. 15.4 Graphical representation of the HCV virus-host (V-H) human interaction network (*red lines*) with black and red nodes representing viral and human proteins, respectively. Blue lines represent H-H interactions (adapted from De Chassey et al. (2008))

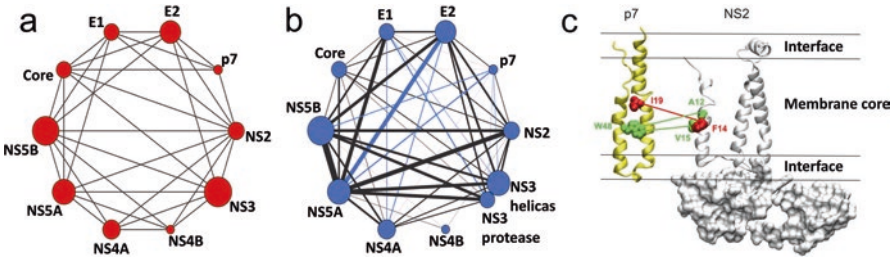

Fig. 15.5 (**a**) Network of reported intraviral HCV PPIs determined experimentally [adapted from Hagen et al. (2014)] compared to (**b**) coevolution links of HCV proteins (Champeimont et al. 2016), where *blue* lines correspond to coevolving links not experimentally reported; (**c**) predicted p7-NS2 interaction between F14 of NS2 and I19 in TM1 of p7 (red line), compared to experimentally reported interactions based on NMR (Cook et al. 2013) between NS2 A12 and V15 and W48 in p7 TM2 (*green* lines) [adapted from Champeimont et al. (2016)]

Coronavirus Viroporins

Coronaviruses (CoV) are vertebrate pathogens which cause human respiratory diseases that typically affect the respiratory tract and gut. CoVs belong to the family *Coronaviridae*, subfamily *Coronavirinae,* and are distributed into four genera α, β, γ, and δ (Enjuanes et al. 2000). While α-CoVs and β-CoVs circulate in mammalian hosts, γ-CoVs and δ-CoVs mainly infect birds. For example, β-CoVs include the murine hepatitis virus (MHV), whereas γ-CoVs include the avian infectious bronchitis virus (IBV). The first coronavirus was isolated in 1937 (IBV), whereas the first human coronavirus (HCoV) was identified in the 1960s. In humans, disease caused by coronaviruses ranges from mild to really severe, e.g., the recent severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS).

SARS-CoV appeared in 2002 causing ~10,000 human infections, with a 10% mortality rate (Holmes 2003). MERS coronavirus (MERS-CoV) emerged about 10 years later, and to date (as of July 2017) almost 2040 cases of infection and 712 deaths have been confirmed (http://www.who.int/emergencies/mers-cov/en/), i.e., a mortality of ~35%. Currently, no effective licensed prevention nor treatment exists against coronavirus infection (Lou et al. 2014), although live attenuated vaccines and fusion inhibitors are promising strategies.

CoVs have nonsegmented, exceptionally long genomes (up to 32 kb). One third of the genome hosts the ORFs for structural proteins, i.e., spike (S), envelope (E), membrane (M), and nucleoprotein (N). This part of the genome also encodes other so-called “accessory” proteins, which vary in number and sequence even among CoVs belonging to the same lineage (Enjuanes et al. 2008), e.g., from one in HCoV-NL63 to eight in SARS-CoV. The remaining two thirds of the genome encode nonstructural genes, with open reading frames ORF1a and ORF1b that produce polyproteins pp1a and pp1ab. These are then processed into 16 nonstructural

proteins (nsp1 to 16); see Su et al. (2016) for recent general overview of coronaviruses and Forni et al. (2017) for the molecular evolution of HCoVs. In the case of SARS-CoV, the genome is predicted to encode 14 functional open reading frames, leading to the expression of up to 30 structural and nonstructural protein products.

E Viroporin

The E proteins are 76–109 amino acids long with one TMD (Torres et al. 2006; Li et al. 2014a; To et al. 2017), a short lumenal N-terminus and a longer cytoplasmic C-terminal tail (Nieto-Torres et al. 2011) which in SARS E protein tends to form β-structure in isolation, but it is mainly helical in the context of a full length protein (Li et al. 2014a). E proteins are localized particularly in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC), where virus morphogenesis and budding occurs. E protein forms homopentameric channels with poor ion selectivity (Verdia-Baguena et al. 2012). Only the TMD of SARS-CoV E (E-TM) has been characterized in some detail, in lipid membranes (Torres et al. 2006) and in DPC micelles (Pervushin et al. 2009).

SARS-CoV E protein is a virulence factor critical for viral pathogenesis, as SARS-CoV lacking the E gene (rSARS-CoV-ΔE) is attenuated in vivo (DeDiego et al. 2007). Mutations N15A and V25F abolish channel activity in vitro (Torres et al. 2007) and led to attenuation when introduced in a recombinant SARS-CoV (Nieto-Torres et al. 2014). The latter authors showed that channel activity is important for inflammasome activation and elevated production of the pro-inflammatory cytokine IL-1β. SARS-CoV E also regulates host stress response and apoptosis (DeDiego et al. 2011) and improves viral fitness. The importance of the E protein in coronavirus pathogenesis has led to the development of live attenuated vaccines based on E-deleted, E-truncated, or E-mutated virions, e.g., Regla-Nava et al. (2015). In general, E protein plays important roles in coronavirus assembly and morphogenesis, although E protein is not necessary to obtain infectious SARS-CoV (DeDiego et al. 2008).

E-M Interaction

E protein is a known binder of M protein, the most abundant protein component of the virion and the membrane protein responsible for its shape. M protein has three predicted TMDs and a large C-terminal extramembrane domain exposed to the cytoplasm or the interior of the virion. It is this domain that forms contacts with the C-terminal tail of the E protein, although TMD interactions are also likely (Lim and Liu 2001; Hogue and Machamer 2008). These interactions occur at the ERGIC, the budding compartment of the host cell. Since M-M interactions are major drivers of

viral envelope formation, these contacts are likely to be important for particle assembly. The E-M interaction has long been reported in infectious bronchitis virus (IBV) and mouse hepatitis virus (MHV) by coimmunoprecipitation in virus-infected or virus-transfected cells (Corse and Machamer 2003; Lim and Liu 2001; Maeda et al. 1999) and was proposed to be crucial for the formation of virus-like particles (VLPs) and virions. In SARS-CoV, coexpression of M and E in a baculovirus expression system was sufficient for the assembly of VLPs (Ho et al. 2004). The deletion of the E gene (ΔE) in the murine coronavirus, the mouse hepatitis virus (MHV), produced revertants where the M gene appeared to have been duplicated, creating new variants of M protein that lacked most of its C-terminal cytoplasmic tail (Kuo and Masters 2010). These results suggested a role for E proteins in "dispersing or de-aggregating" M protein during packaging.

Other CoV Viroporins Although the most studied viroporin in CoVs is the envelope (E) protein, other viroporins have been found in an accessory gene present in all CoVs, between the S and E gene loci. In SARS-CoV (SARS-ORF3a) and in HCoV-229E (229E–ORF4a), these proteins form ion channels (Zhang et al. 2014; Lu et al. 2006), whereas HCoV-NL63-ORF3 has also been proposed to be a viroporin (Zhang et al. 2015). In HCoV-OC43, this genomic segment encodes ORF5, which has been reported to facilitate virion morphogenesis (Zhang et al. 2015). The latter has only a single TMD, in contrast with SARS-CoV ORF3a, HCoV-229E ORF4a, and HCoV-NL63 ORF3.

3a Viroporin SARS-CoV 3a protein is a 274-amino-acid-long protein with three TMDs and forms homotetrameric complexes that have ion channel activity (Lu et al. 2006). 3a protein has a cysteine-rich domain (residues 127–133) responsible for homo- and hetero-dimerization (Lu et al. 2006). In addition to a Yxx domain and a diacidic domain, it has a C-terminal domain (Tan et al. 2006). The C-terminal domain has RNA-binding activity (Sharma et al. 2007). Protein 3a is suggested to play a structural role in the SARS-CoV life cycle, since it interacts with S, E, and M proteins (Tan et al. 2004) and it is incorporated into newly packaged matured SARS-CoV virions (Shen et al. 2005; Ito et al. 2005). Also, it regulates various cellular responses of host cells, e.g., the upregulation of fibrinogen gene expression (Tan et al. 2005), and the increase of IL-8 and NF-B promoter activities (Kanzawa et al. 2006), possibly through its RNA-binding activity (Sharma et al. 2007). SARS-CoV 3a protein induces caspase-dependent apoptosis both in vivo and in vitro (Wong et al. 2005).

Intraviral Interactions

Two yeast-two-hybrid (Y2H) studies have been conducted to study intraviral SARS-CoV protein interactions (von Brunn et al. 2007; Pan et al. 2008), although only a few of these interactions have been verified. Von Brunn et al. (von Brunn et al. 2007)

reported interactions of E protein with the nonstructural proteins nsp1, nsp8, and nsp11, as well as with the accessory proteins ORF3b, ORF7b, and ORF9b, whereas ORF3a interacted with M and S. However, not all these interactions were confirmed by coimmunoprecipitation in mammalian cells. Overall, however, only 13% of the intraviral SARS interactions known at that time were identified. This is likely due to the bias of Y2H against membrane proteins, which prevents the transfer of expressed prey and/or bait fusion proteins to the nucleus to activate transcription. Pan et al. (Pan et al. 2008) also reported a genome-wide analysis of intraviral PPIs in SARS-CoV replication, using a mammalian two-hybrid system screen, although only two interactions of E and 3a were found here. In comparison with a similar screen in yeast, native posttranslational modifications and folding should be present, but the two methods share a similar limitation in terms of bias against membrane proteins.

Later, a tandem affinity purification (TAP) study coupled to mass spectrometry (Álvarez et al. 2010), using dual-tagged SARS-CoV E protein in infected cells as bait, identified viral proteins nsp3, S, and M and host proteins dynein heavy chain, fatty acid synthase, aminopeptidase puromycin sensitive, phosphofructokinase platelet, tubulin alpha and beta, actin beta, transmembrane protein 43, and lactate dehydrogenase A as binders. Nsp3 is the largest nonstructural protein of SARS-CoV (1922 amino acids) which is proposed to act as a replication/transcription scaffolding protein (Imbert et al. 2008). Interaction with nsp3 was confirmed by coimmunoprecipitation and was localized to one of the nsp3 seven domains, i.e., the N-terminal acidic domain (nsp3a), that has a ubiquitin-like fold (Serrano et al. 2007). Colocalization of E and nsp3 in the cytoplasm of the infected cell suggested nsp3 may bring E protein into the vicinity of the replication/transcription complex.

The PLpro domain of nsp3 has deubiquitinating activity (Lindner et al. 2005) and might act to protect the viral replication complex from proteasomal degradation via deubiquitination. The authors proposed that E-nsp3 interaction could control ubiquitination of E protein during infection. Interaction between nsp3a and SARS-CoV E was shown to involve residues in the C-terminal domain of the latter (Li et al. 2014a).

Host Interactions

More recently, two Y2H studies searched for host interacting partners using the C-terminal tail of SARS-CoV E as a bait (Teoh et al. 2010; Jimenez-Guardeño et al. 2014). The first of these reported the protein associated with *Caenorhabditis elegans* lin-7 protein 1 (PALS1) as a binder (Teoh et al. 2010). PALS1 is a tight junction-associated protein and part of a complex that maintains epithelial cell polarity (Fig. 15.6). Alterations of lung epithelia integrity were consistent with E protein hijacking PALS1 to the ERGIC/Golgi region. The E-PALS1 interaction is mediated by (i) a Postsynaptic density protein-95/Discs Large/Zonula occludens-1 (PDZ) domain present in PALS1 and (ii) the last four C-terminal residues of E protein which represent a putative type II PDZ-binding motif (PBM) (Harris and Lim 2001).

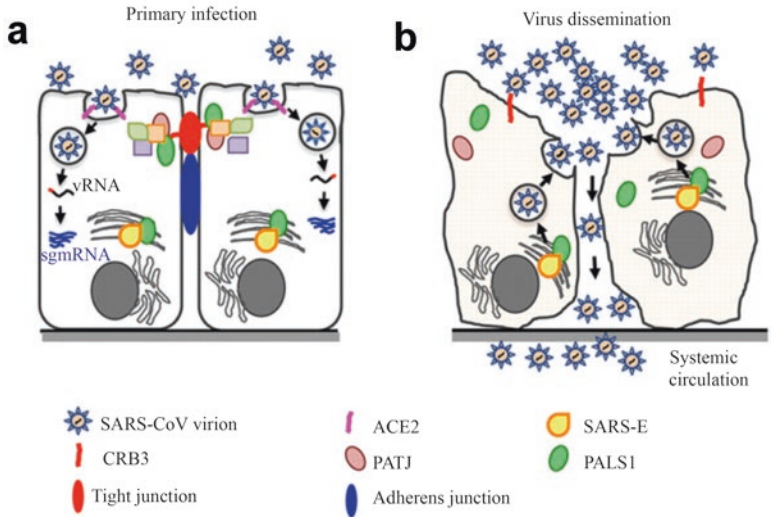

Fig. 15.6 Model of the potential consequences of SARS-CoV infection on polarity and intercellular junctions formed by alveolar epithelial cells. (**a**) The inner surface of human lung alveolae is lined with a monolayer of polarized epithelial cells. Components of CRB and PAR polarity complexes, including PALS1, are shown close to the apical domain. During infection, structural proteins accumulate in the ERGIC compartment, where SARS-CoV E could bind to PALS1 to disrupt its trafficking to the tight junction; (**b**) disruption of the tight junction and virus dissemination [adapted from Teoh et al. (2010)]

PDZ domains are ~80–90 amino acids long and typically bind the C-terminal tails of proteins, although internal binding sites have also been reported [reviewed in Ye and Zhang (2013)]. They are usually found in signaling proteins that alter signaling pathways, with over 250 nonredundant PDZ domains being recognized in the human proteome (Wang et al. 2010).

The C-terminal tail of SARS-CoV E protein, which includes the proposed PBM, forms a random coil secondary structure (Li et al. 2014a) in a variety of environments. However, PBMs usually fold as β-strands (Ye and Zhang 2013), which suggests that a β-structure conformation may be induced by target binding. Another similar Y2H study that used the same bait described a similar PDZ domain-containing binder, the syndecan-binding protein (syntenin) (Jimenez-Guardeño et al. 2014). Syntenin is a scaffolding protein that can initiate a signaling cascade resulting in the phosphorylation and activation of p38 mitogen-activated protein kinase (p38-MAPK), leading to expression of pro-inflammatory cytokines. The authors showed that the proposed C-terminal PBM in SARS-CoV E protein is a determinant of virulence. Since SARS-CoV-infected patients show an exacerbated inflammatory response that leads to epithelial and endothelial damage, edema, and acute respiratory distress syndrome (ARDS), the disruption of this pathway may have therapeutic implications. Overall, an involvement of this PBM in E protein in epithelial integrity and inflammatory responses is likely. Several other viruses, e.g., influenza A virus or human papillomavirus, have been described to enhance

pathogenesis through viral proteins containing PBMs [reviewed in Javier and Rice (2011)], which probably constitute a common viral strategy.

CoV E proteins may also interact with, and modulate, host channels to support the virus life cycle. In *Xenopus* oocytes, it has been shown that coexpression of SARS-CoV E with human epithelial sodium transporter (ENaC) reduced amiloride-sensitive current through PKC activation followed by reduction of ENaC surface levels (Ji et al. 2009). A similar direct or indirect inhibitory effect on other endogenous channels was proposed from patch-clamp experiments using SARS-CoV E-transfected cells (Nieto-Torres et al. 2011). For IBV E, interaction with endogenous channels or SNAREs has been suggested to justify the Golgi complex rearrangement in response to IBV E expression (Ruch and Machamer 2011), although this observation may also involve the IBV E channel itself. For example, ion homeostasis at the Golgi could affect Na^+/H^+ exchangers that are critical for maintaining low luminal pH. Interactions of viroporins with Golgi channels or transporters are largely unexplored in the viroporins field, but notable cases have been already reported. For example, oncogenic protein E5 from papillomavirus (Wetherill et al. 2012) is able to bind the 16 K subunit of the lumen-acidifying V-ATPase (Goldstein et al. 1991), preventing assembly of the pump and leading to alkalinization of the Golgi lumen (Schapiro et al. 2000).

The Respiratory Syncytial Virus Small Hydrophobic Protein (RSV-SH)

Human respiratory syncytial virus (hRSV) belongs to the *Paramyxoviridae* family in the pneumovirus genus. This enveloped virus has a negative-sense single-strand RNA genome 15.2 kb long that encodes 10 sub-genomic mRNAs and 11 proteins (Fields et al. 2013). These 11 proteins include three membrane proteins accessible to the surface of the virion: the two that generate most RSV-neutralizing antibodies, fusion (F) and attachment (G), and the small hydrophobic (SH) protein.

RSV affects more than 30 million children below 5 years old and is the leading cause of bronchiolitis and pneumonia in infants and elderly (Dowell et al. 1996). Disease caused by RSV is responsible for 200,000 deaths worldwide which mostly occur in developing countries. hRSV exists as two antigenically distinct subgroups, A and B, both capable of inducing severe lower respiratory tract (LRT) disease in humans (Hall et al. 1990).

Although the virus was isolated more than half a century ago, no effective licensed treatment or vaccine is available for the general population, despite promising RSV vaccine candidates in clinical trials. Palivizumab is a humanized monoclonal antibody (IgG) directed against the F protein that is recommended for infants <2 years old with high risk. However, it is not effective therapeutically and is only moderately effective at preventing infection. Since it costs $4500 per treatment course (Weiner et al. 2011), its use is limited to a small fraction of patients worldwide. The only licensed drug for therapeutic use is a nucleoside analog which has limited efficacy.

SH Viroporin

The SH protein in hRSV is only 64 (subgroup A) or 65 (subgroup B) amino acids long, but its sequence is well conserved, especially the N-terminal extramembrane domain (Tapia et al. 2014). It has a single TM α-helical hydrophobic region, with C- (lumenal or extracellular) and N- terminal (cytoplasmic) extramembrane domains (Collins and Mottet 1993). The N-terminal cytoplasmic domain forms a short α-helix (residues 5–14) (Fig. 15.7a), almost coincident with a "10-residue" conserved sequence between hRSV and MuV SH protein sequences. SH proteins in MuV, PIV5, and JPV have extremely short lumenal domains (nine, two, and ten residues, respectively) compared with their much longer N-terminal cytoplasmic domains, which are likely involved in PPIs. The C-terminal extramembrane domain forms an extended β-hairpin. In bicelles, the α-helix of the TMD extends up to residue His-51 (Li et al. 2014b), resulting in both protonatable residues of SH protein, His-22, and His-51, oriented toward the lumen of the channel.

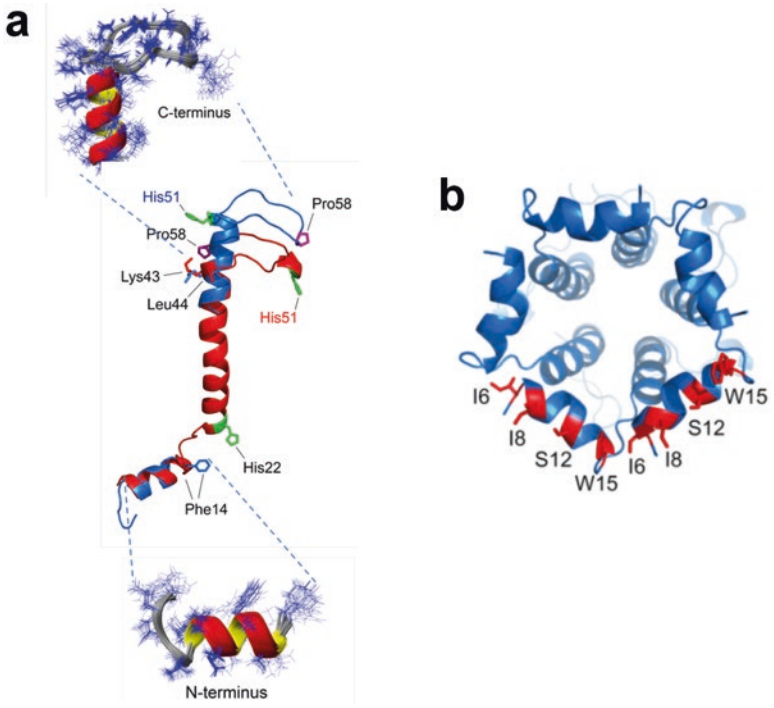

Fig. 15.7 Structural model of SH protein monomer. (**a**) Comparison of models of monomeric SH protein obtained in micelles (*red*) and in bicelles (*blue*), with residues prolonging the TM domain up to His-51 (Li et al. 2014b); (**b**) residues in SH involved in interaction with BAP31; N-terminal cytoplasmic helix of SH protein, with residues perturbed (*red*) after addition of BAP31 cytoplasmic domain to labeled SH protein in detergent micelles (Li et al. 2015)

SH protein forms homo-oligomers (pentamers), and this oligomeric form is responsible for ion channel (IC) activity (Gan et al. 2012; Gan et al. 2008) that has poor ion selectivity. In infected cells, most SH protein accumulates at the membranes of the Golgi complex, but it is also found in the ER or plasma membrane (Rixon et al. 2004). SH has potential glycosylation sites in both the C- and N-terminal domains (Collins et al. 1990). In infected cells, the SH protein of strain A2 accumulates in four different forms (Olmsted and Collins 1989; Collins et al. 1984; Collins and Mottet 1993), but the most abundant is a full-length unglycosylated form. The G protein forms G-F and G-SH complexes, but direct interactions between SH and F have not been observed (Low et al. 2008).

SH and apoptosis. It has been proposed that SH protein blocks apoptosis through inhibition of the TNF-α pathway (Fuentes et al. 2007), but the mechanism of this inhibition is not clear. A similar anti-apoptotic effect of SH protein has been reported for other members of the *Paramyxoviridae* family that encode SH proteins, e.g., mumps virus (MuV) and the parainfluenza virus 5 (PIV5).

Incidentally, an anti-apoptotic effect has also been noted for other similar viral channels (viroporins), e.g., E5 in the human papillomavirus type 16 (HPV-16) (Kabsch et al. 2004), or the envelope (E) protein, a viroporin in the severe acute respiratory syndrome (SARS) virus (DeDiego et al. 2011).

SH and the Inflammasome

SH protein is also involved in inflammasome regulation, but the mechanism involved is not known. Indeed, some authors have proposed that RSV SH has a role in regulation of the NLRP3 inflammasome (Russell et al. 2015). The latter is "primed" after the recognition of viral genomic RNA (vRNA) by pattern recognition receptors (PRRs) and subsequent activation of NF-kB. This priming involves the expression of inflammasome components, e.g., NLRP3 and inactive procaspase-1 (Elliott and Sutterwala 2015). Various virus-induced damage-associated molecular patterns (DAMPs) induce the assembly and activation of the NLRP3 inflammasome. This leads to processing of procaspase-1 into active caspase-1, which in turn cleaves inactive pro-IL-1β into the mature form IL-1β. The latter is a potent pro-inflammatory cytokine crucial in resolving infectious processes.

Various viruses can activate the inflammasome by disrupting ion homeostasis through the expression of viroporins. For example, influenza A virus (IAV) activates NLRP3 as a result of H^+ or ion flux from Golgi mediated by the M2 channel (Ichinohe et al. 2010). The 2B protein in picornaviruses induce NLRP3 cytoplasmic relocalization and inflammasome activation in an intracellular Ca^{2+}-mediated manner (Ito et al. 2012), while a similar mechanism has been proposed for SARS-CoV E (Nieto-Torres et al. 2015). The latter triggered inflammation in the lungs of mice, leading to epithelial cell damage and death (Nieto-Torres et al. 2014), and this was correlated to high levels of mature IL-1β in the airways of infected animals.

Similarly, RSV SH protein has been suggested to activate the NLRP3 inflammasome through its IC activity and ion leakage from the Golgi (Triantafilou et al. 2013), similar to the mechanism proposed for SARS-CoV E (Nieto-Torres et al. 2015). Another study (Russell et al. 2015) proposed that IL-1β is overproduced when SH is absent from RSV. The study also showed attenuation in mice when infected with RSV ΔSH. That deletion of RSV SH leads to an increase in IL-1β is also supported by studies in bovine RSV (bRSV), where a ΔSH vaccine strain induced higher levels of IL-1β in the lungs of infected cattle (Taylor et al. 2014). Consistent with this, lung macrophages infected with RSV did not lead to increased IL-1β, although other pro-inflammatory cytokines were overexpressed (Ravi et al. 2013). Overall, it has been proposed (Russell et al. 2015) that SH protein blocks IL-1β production, preventing the clearance of infected cells. Indeed, blockade of IL-1β prior to infection increased the viral load, supporting the idea that SH might enable immunomodulation. The interaction between SH and G protein has also been shown previously to have an immunomodulatory role (Polack et al. 2005).

Although in cell culture RSV ΔSH is still viable, grows to similar titer to wild-type RSV, and still forms syncytia, SH-deleted RSV (RSV ΔSH) is significantly attenuated in a variety of hosts (Taylor et al. 2014; Bukreyev et al. 1997; Russell et al. 2015). Indeed, in the last few years, one of the leading RSV LAVs have included, among other modifications, a deletion in the SH gene (Karron et al. 2005). The cause of attenuation is not known, although it may have to do with effective transmission of the virus (Bukreyev et al. 1997).

A transcriptome analysis comparing RSV with and without SH protein could help to decipher the role of SH during infection and the cause of attenuation in vivo and to associate these responses to specific SH domains or features.

Host Interactions

Recently, a membrane-based yeast two-hybrid system (MbY2H) was used to identify a cellular binding partner of hRSV SH protein, the B-cell receptor-associated protein 31 (BAP31) (Li et al. 2015), in a human lung cDNA library. BAP31 is a membrane protein located at the ER that has an essential role in sorting newly synthesized membrane proteins. Additionally, BAP31 has a cytoplasmic C-terminus with two coiled coils (Quistgaard et al. 2013), one of them containing a variant of the death effector domain (vDED) flanked by two caspase-8 cleavage sites. This domain is excised upon activation of caspase-8 to produce a fragment p20, known to function as a proapoptotic factor (Breckenridge et al. 2003). This interaction was confirmed using co-transfection, pull-down assay and immunofluorescence colocalization, and also using endogenous BAP31 and was localized to the first N-terminal 44 residues (Li et al. 2015). When ^{15}N–labeled SH protein was titrated with cytoplasmic C-terminal domain of human BAP31, major shifts were observed at residues I6, I8, S12, and W15 (Fig. 15.7b). It can be hypothesized that this interaction could interfere with the interaction between BAP31 and caspase-8, blocking

the cleavage sites and preventing conversion to the pro-apoptotic form of BAP31, i.e., p20, thus delaying apoptosis. Incidentally, the viroporin E5 from the high-risk human papillomavirus HPV-16 and HPV-31 was also found to interact with BAP31, where it is similarly thought to regulate apoptosis in addition to its roles in immunomodulation (Regan and Laimins 2008) (see below).

Intraviral Interactions

The interaction between RSV SH and G proteins has been reported previously in infected cells (Low et al. 2008; Rixon et al. 2005), although its significance is not yet clear. F protein seems to be the main determinant of host cell specificity during virus entry, and both F and G proteins are able to bind heparin sulfate, the putative cell receptor for RSV (Kargel et al. 2001). However, a tri-component complex between SH, G, and F proteins was not observed (Low et al. 2008). Both G and F proteins have one predicted TMD, and interaction with SH protein can be both through the TMDs or extramembrane domains of the latter.

Until now, all studies to determine the role of SH protein in RSV infection have used wild-type RSV and ΔSH RSV (SH gene deleted) and compared the effects of this deletion on various parameters in infected cells, or in animal models. The effects caused by transfection of SH protein in readouts that depend on, for example, inflammasome activation or apoptosis, have also been explored. However, comprehensive datasets that aim at elucidating the contribution of SH to virulence observed in vivo, and a rationale for the attenuation observed when SH is deleted, are lacking.

The Human Immunodeficiency Virus Viral Protein U (HIV-1-Vpu)

The human immunodeficiency virus type 1 (HIV-1) is an enveloped virus that causes AIDS. It has a single-stranded, positive-sense RNA genome of 9.8 kb which encodes for nine genes: the structural genes *gag*, *pol*, and *env*, the regulatory genes *tat* and *rev*, and additional genes *nef*, *vif*, *vpr*, and *vpu* which encode for accessory proteins. One of these accessory proteins, the Vpu (viral protein U) (Cohen et al. 1988), is an 81-residue small-membrane protein consisting of an N-terminal transmembrane helix and a C-terminal cytoplasmic domain which contains two helices linked by a flexible loop region [reviewed in Opella (2015)] (Fig. 15.8a). Vpu can oligomerize to form cation-selective channels in membranes, although the rationale for this channel activity is not well defined.

Vpu has two primary roles during HIV-1 infection: (i) enhancement of virion release (Terwilliger et al. 1989; Strebel et al. 1988) and (ii) degradation of host CD4 receptor (Willey et al. 1992). Absence of Vpu in infected cells correlates with

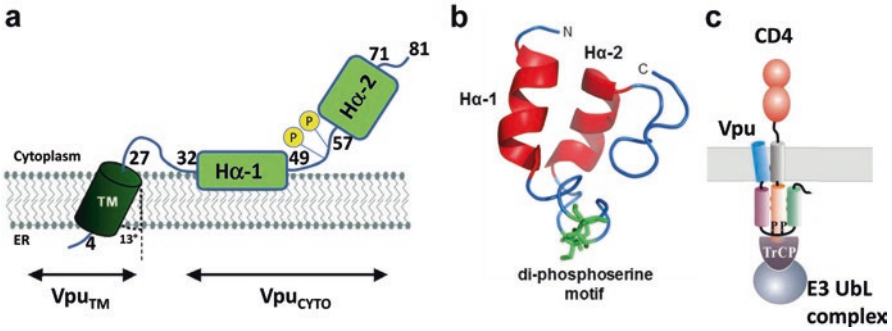

Fig. 15.8 (**a**) Predicted secondary structure of Vpu showing N-terminal TMD (*blue*) and two α-helices of the cytoplasmic (CYTO) domain (*red*). Phosphorylated S52 and S56 are represented as circles (adapted from Dubé et al. (2010)). (**b**) Solution NMR structure of Vpu_{CYTO} in DPC micelles (Protein Data Bank code: 2K7Y). Helix 1 (Ile39-Glu48) and helix 2 (Leu64-Arg70) are shown as ribbons. β-TrCP-binding DSGxxS motif is in blue. Side chains of phosphorylated serines are shown as balls and sticks. Vpu_{CYTO} residues showing substantial chemical shift changes upon addition of 1 mM CD4mut are in red (adapted from Singh et al. (2012)); (**c**) Vpu-mediated degradation of CD4. Binding of Vpu to CD4 is mediated by the cytoplasmic helices and TM helices. Vpu has two conserved phosphoserines which constitute a binding motif for β-TrCP, leading to assembly of an E3 ubiquitin ligase (UbL) complex that results in CD4 ubiquitination and proteasomal degradation (modified from Strebel (2014))

reduction of viral release and intracellular accumulation of HIV-1 viral proteins (Klimkait et al. 1990). The host protein BST-2 is a restriction factor of HIV-1 release, and its activity can be neutralized by Vpu (Neil et al. 2008; Van Damme et al. 2008).

Host Interactions A number of host factors have been reported to bind Vpu, including the immunoreceptors major histocompatibility complex class I (MHC-I) (Kerkau et al. 1997), CD1d (Moll et al. 2010), NK-T-B-antigen (NTB-A) (Shah et al. 2010), poliovirus receptor (PVR) (Matusali et al. 2012) and human leukocyte antigen C (HLA-C) (Apps et al. 2016), C-C chemokine receptor type 7 (CCR7) (Ramirez et al. 2014) and CD62L (Vassena et al. 2015), tetraspanins (Haller et al. 2014; Lambelé et al. 2015), K^+ channel TASK-1 (Hsu et al. 2004), metabolic transporter, sodium-coupled neutral amino acid transporter 1 (SNAT1) (Matheson et al. 2015), and the most recently reported intercellular adhesion molecule 1 (ICAM-I) (Sugden et al. 2017). However, the most important binders are CD4 and BST-2.

Vpu-Mediated Degradation of CD4

The host CD4 is a cell surface receptor critical for HIV-1 entry into target cells by endocytosis, but its expression at the cell surface prevents the release of infectious virions from infected cells. To counteract this host defense mechanism, viral Vpu,

Env, and Nef downmodulate CD4 surface expression [see review in Doms and Trono (2000)]. Vpu interacts with newly synthesized CD4 at the ER to mediate CD4 degradation (Willey et al. 1992). Vpu-mediated degradation of CD4 requires physical interaction between the two proteins. In CD4, the interaction domain has been mapped to a specific motif (L_{414}SEKKT$_{419}$) and a membrane-proximal α-helix (Bour et al. 1995). For Vpu, residues in both cytoplasmic α-helices of Vpu experienced chemical shift perturbations upon CD4 binding (Fig. 15.8b) (Singh et al. 2012), although involvement of the TMDs of both proteins has also been suggested (Magadán and Bonifacino 2012; Do et al. 2013). However, the scrambling or replacement of the whole Vpu TMD appears to have no effect on Vpu-mediated degradation of CD4 (Willey et al. 1994; Schubert et al. 1996), whereas a single amino acid substitution at the TMD, W22 L, abolished CD4 degradation but did not disrupt the CD4-Vpu interaction (Magadán and Bonifacino 2012), suggesting that TM interactions between CD4 and Vpu may function beyond the expected role of stabilizing the protein complex for CD4 degradation.

Overall, it has been proposed that Vpu acts as an adapter protein to link CD4 to the host ubiquitin-proteasome machinery for degradation. This binding triggers the recruitment of the host beta-transducin repeat-containing protein (β-TrCP). A conserved di-phosphoserine motif (D_{51}SGxxS_{56}) located within the loop region that connects the two cytoplasmic α-helices of Vpu is necessary for this process. Interaction of the phosphoserines in Vpu with the WD boxes of β-TrCP enables the formation of a CD4-Vpu-β-TrCP ternary complex (Margottin et al. 1998), bringing CD4 and other components of the $SCF^{\beta\text{-TrCP}}$ E3 ubiquitin ligase complex (Skp1 and Cullin1) in close proximity to facilitate the *trans*-ubiquitination of the CD4 cytosolic tail (Binette et al. 2007) on lysine and serine/threonine residues (Magadán et al. 2010) and subsequently its transportation to the cytosol for degradation (Fig. 15.8c).

Vpu-Mediated Antagonism of BST-2

Vpu enhances virus dissemination by antagonizing the host BST-2, a host restriction factor with antiviral capabilities [reviewed in Simon et al. (2015)]. BST-2 is an interferon-inducible type II integral membrane protein located at the budding site of HIV-1. BST-2 has an N-terminal cytoplasmic domain, a TMD, followed by a coiled-coil ectodomain and finally a C-terminal glycosyl-phosphatidylinositol (GPI) membrane anchor. Its unusual topology enables it to tether virions by inserting, preferentially its GPI anchor, into the envelope of assembling virion particles, while itself remains embedded in its host cell membrane (Venkatesh and Bieniasz 2013; Neil et al. 2006).

It has been proposed that Vpu engages BST-2 through interaction between their respective TMDs, with involvement of Vpu's A14, W22, and A18 (Vigan and Neil 2010) and BST-2's I34, L37, and L41 (Kobayashi et al. 2011). An NMR study described an antiparallel interaction between Vpu and BST-2 TMDs in DHPC

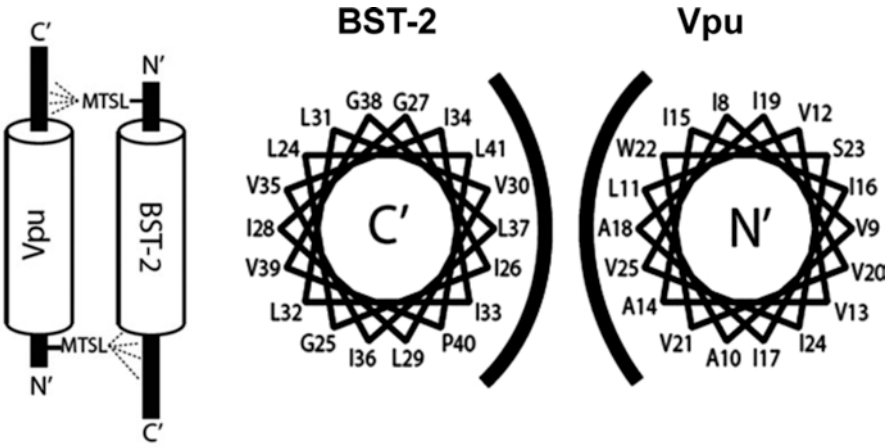

Fig. 15.9 *Left*, the spin label (MTSL) at the N-terminus of Vpu selectively decreased the intensity of C-terminal residues of BST-2, whereas the spin label at the N-terminus of BST-2 selectively decreased the intensity of C-terminal residues of Vpu; *right*, helical wheel diagrams of the BST-2 and Vpu TMDs. The TMDs of BST-2 and Vpu are depicted in their anti parallel orientation (adapted from Skasko et al. (2012))

micelles, in an orientation where A18 of Vpu faces L37 of BST-2 (Skasko et al. 2012) (Fig. 15.9). The conserved residues within the Vpu membrane-proximal cytoplasmic hinge region (E_{28}YRKIL$_{33}$) have also been found to be important for Vpu-mediated BST-2 antagonism (Lukhele and Cohen 2017).

The mechanism of BST-2 neutralization by Vpu appears to be multifaceted and under debate, although the key mechanism appears to be the direct displacement of BST-2 from the virus assembly sites at the plasma membrane (McNatt et al. 2013). Accordingly, a C-terminal Trp residue at the Vpu cytoplasmic tail has been reported to contribute to this displacement of cell surface BST-2 (Lewinski et al. 2015). Other proposed mechanisms have been proposed, e.g., Vpu can disrupt intracellular BST-2 trafficking by sequestering both newly synthesized and recycling BST-2 within intracellular compartments such as the TGN (Dubé et al. 2010; Hauser et al. 2010). This effectively blocks the resupply of BST-2 to the plasma membrane and thereby reduce BST-2 surface density (Dubé et al. 2011). Vpu-mediated BST-2 mistrafficking has been reported to involve the host clathrin-dependent membrane trafficking pathways which are mediated by clathrin adaptor protein (AP) complexes (Kueck and Neil 2012; Lau et al. 2011). It was proposed that Vpu is able to hijack the clathrin-dependent trafficking machinery via a mimicked canonical acidic di-leucine sorting motif (E_{59}xxxLV$_{64}$) within its second cytoplasmic α-helix to recruit the AP complexes, forming a Vpu-BST-2-AP ternary complex (McNatt et al. 2013; Jia et al. 2014; Kueck et al. 2015). In addition, the conserved di-phosphoserine motif (D_{51}SGxxS$_{56}$) in Vpu may also be required for this recruitment (Kueck et al. 2015).

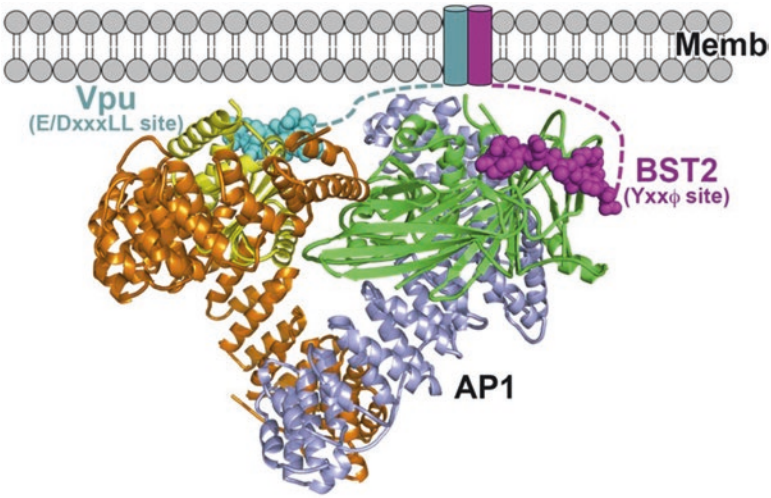

Fig. 15.10 Schematics of Vpu hijacking of AP1 to target BST2. AP1 is colored by subunit (β1 in gray, γ in *orange*, μ1 in *green*, and σ1 in *yellow*). Vpu_{CYTO} (*cyan*) binds to the acidic di-leucine-binding pocket of γ/σ1, and BST-2_{CYTO} (*magenta*) binds to the tyrosine-binding pocket in μ1. Transmembrane helices are represented by cylinders (adapted from Jia et al. (2014))

A crystal structure of a Vpu_{CYTO}-BST-2_{CYTO} fusion protein in complex with the AP1 core has been solved (Jia et al. 2014). In this model, the cytoplasmic domains of Vpu and BST-2 do not interact directly. Instead, Vpu seems to act as a chaperone to enhance binding of AP1 to BST-2. Stability of this complex is achieved by pair-wise binary interactions between Vpu and BST-2 TMDs and between Vpu and BST-2 cytoplasmic domains to several parts of AP1 (Fig. 15.10). Thereafter, the Vpu-BST-2 complex is thought to proceed through the clathrin-mediated trafficking pathway for β-TrCP-dependent ubiquitination of BST-2 before subsequent ESCRT-mediated endo-lysosomal degradation.

Recently, it has been reported that Vpu may hijack the function of the host actin cross-linking regulator filamin A (FLNa) during its quest of BST-2 modulation (Dotson et al. 2016). Vpu may also exploit an LC3-associated noncanonical autophagy pathway to restrict BST-2 (Madjo et al. 2016). The refinement of current models, together with the discovery of additional host and/or intraviral factors involved, will ultimately form a complete and accurate picture of Vpu-mediated BST-2 antagonism.

The Polyomavirus JC Agnoprotein

Polyomaviruses are small, non-enveloped DNA viruses with their closed circular genome packaged within an icosahedral viral capsid. Progressive multifocal leukoencephalopathy (PML), a deadly demyelinating disease of the brain, is attributed to

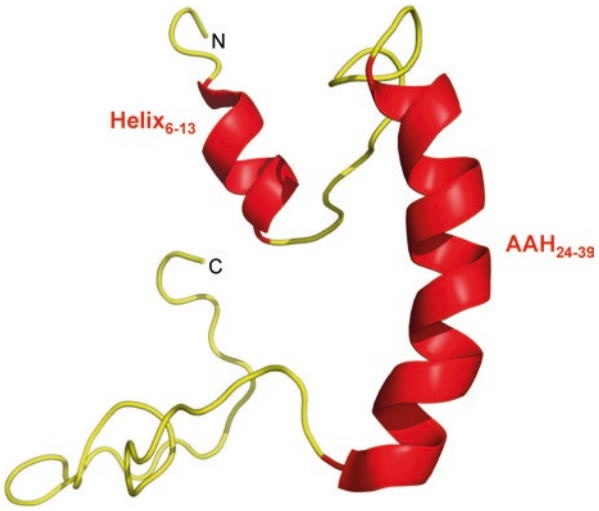

Fig. 15.11 NMR structure of agnoprotein contains two main α-helical structures (*red*) and two unstructured regions (*yellow*) [adapted from Coric et al. (2017)]

the human polyomavirus JCV (John Cunningham virus). JCV encodes for a small and highly basic protein called agnoprotein which has important regulatory roles in the JCV replication cycle. Besides JCV, the agnoprotein can also be found in other polyomaviruses including BK virus (BKV) and simian virus 40 (SV40) (Sariyer et al. 2011). The 71-residue agnoprotein has a central hydrophobic region which has been reported to form an amphipathic α-helix (Lys23-Phe39) (Coric et al. 2014). This helix is characteristically rich in Leu/Ile/Phe, which is required for protein stability and oligomerization (Saribas et al. 2013). A recently solved NMR structure in organic solvent has revealed a second α-helix, albeit minor, spanning residues Leu6-Lys13 (Fig. 15.11) (Coric et al. 2017).

Agnoprotein demonstrates several key features which are commonly shared among viroporins (Suzuki et al. 2010a; Suzuki et al. 2013; Suzuki et al. 2010b), e.g., it is a membrane protein that associates into homo-oligomers that increased membrane permeability leading to influx of extracellular Ca^{2+} and enhancement of virus release. In addition, agnoprotein-deleted mutants have defective virion release and viral propagation.

Intraviral and Host Interactions The JCV agnoprotein has been shown to interact with a number of viral proteins: large T-antigen (LT-ag) (Safak et al. 2001), small t-antigen (St-ag) (Sariyer et al. 2008), HIV-1 Tat (Kaniowska et al. 2006), and capsid protein VP1 (Suzuki et al. 2012). It also interacts with cellular proteins, including the Y-box-binding factor 1 (YB-1) (Safak et al. 2002), tumor suppressor p53 (Darbinyan et al. 2002), tubulin (Endo et al. 2003), DNA damage repair protein Ku70 (Darbinyan et al. 2004), fasciculation and elongation protein zeta 1 (FEZ1) (Suzuki et al. 2005), heterochromatin protein 1 alpha (HP-1α) (Okada et al. 2005), protein phosphatase 2A (PP2A) (Sariyer et al. 2008), and the adaptor protein complex 3 (AP3) δ subunit (Suzuki et al. 2013).

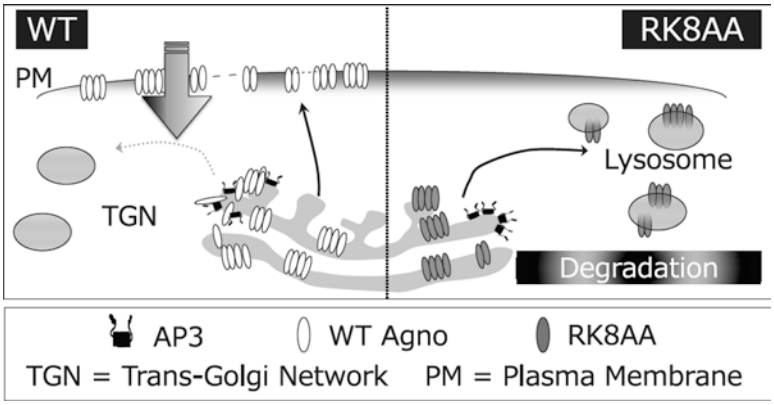

Fig. 15.12 Involvement of AP-3 in membrane permeabilization and virion release involving WT (*left*) and mutant RK8AA (*right*) agnoprotein. Both WT and RK8AA form homo-oligomers as integral membrane proteins in cytoplasmic organelles. WT disrupts AP3-mediated vesicular trafficking, is translocated to plasma membrane, and functions as a viroporin, resulting in the promotion of virion release. In contrast, the RK8AA mutant fails to bind to AP3D and does not disrupt AP3-mediated vesicular trafficking and is transported to lysosomes and degraded. RK8AA agnoprotein cannot promote virion release and is defective in viral propagation [adapted from Suzuki et al. (2013)]

One of the interesting host factors targeted by the JCV agnoprotein is the AP3 (Suzuki et al. 2013). Interaction of agnoprotein with the δ subunit of AP3 (AP3D) appears to hijack the AP3-mediated intracellular vesicular trafficking to prevent the targeted lysosomal degradation of agnoprotein. This phenomenon is reminiscent of the special features of viroporins such as M2 and Vpu, which also manipulate host trafficking pathways. Agnoprotein is then allowed to be translocated to the plasma membrane to act as a viroporin and promote virion release (Suzuki et al. 2013). Alanine substitutions of two basic residues (Arg8 and Lys9) in the N-terminus of agnoprotein disrupt its viroporin activity (Suzuki et al. 2010a) and also disrupt binding to AP3D, ensuing its transport to the lysosomes and subsequent lysosomal degradation (Fig. 15.12) (Suzuki et al. 2013). These basic residues are part of an ordered helical structure (Coric et al. 2017) and may constitute an important regulatory and/or interaction motif.

Rotavirus NSP4

Rotaviruses are members of the *Reoviridae* family of non-enveloped viruses which consist of segmented, double-stranded RNA genomes surrounded by multiple concentric protein capsids (Coombs 2006). Rotaviruses are a leading cause of severe viral gastroenteritis and dehydrating diarrhea in infants and young children, resulting in a high global child mortality rate of 215,000 in 2013 (in children <5 years old)

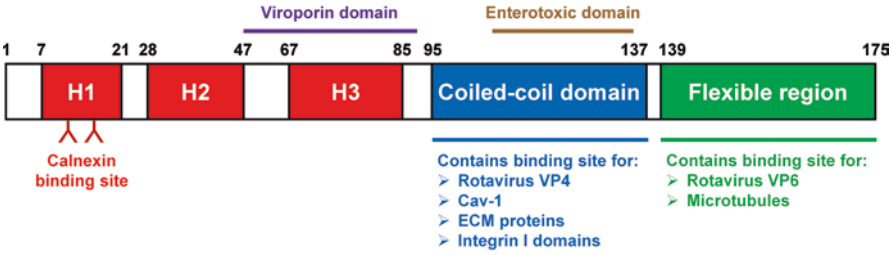

Fig. 15.13 Schematic representation of rotavirus NSP4 structural domains. H1, H2, and H3, hydrophobic domains 1, 2, and 3, respectively. NSP4 viroporin domain (residues 47–90) and enterotoxic domain (residues 114–135) are indicated. Putative binding sites of NSP4 interaction partners are also indicated

(Tate et al. 2016). The rotavirus nonstructural protein 4 (NSP4) is a 175-amino-acid transmembrane ER glycoprotein and the first virus-encoded enterotoxin to be discovered (Ball et al. 1996). Besides its primary ER localization, NSP4 can also be secreted as a soluble enterotoxin (Bugarčić and Taylor 2006) or colocalize with the autophagy protein LC3 in cap-like structures that associate with viroplasms (Berkova et al. 2006).

NSP4 consists of three hydrophobic domains (H1, H2, and H3) followed by a long cytoplasmic region containing a coiled-coil domain (CCD) and a flexible tail region (Estes and Greenberg 2013) (Fig. 15.13). Notably, its distinctive functional domains include (i) an enterotoxic domain (ETD, residues 114–135), which can function as a diarrhea-inducing enterotoxin similar to the full-length protein in young mice (Ball et al. 1996), and (ii) a recently discovered viroporin domain (VPD, residues 47–90) which is made of a penta-lysine domain and the amphipathic helix H3 (Hyser et al. 2010) and exhibits cation-selective channel activity in artificial lipid bilayers (Pham et al. 2017).

An alteration in cellular calcium homeostasis is critical for rotavirus replication and cytopathogenesis, and this has been correlated with the NSP4 viroporin [see review in Hyser and Estes (2015)]. Earlier studies reported that NSP4 colocalize with the autophagosome marker LC3 in "cap-like structures" associated with viroplasms (Berkova et al. 2006), sparking interest of whether the host autophagy machinery is manipulated during rotavirus infection. Indeed, NSP4 appears to orchestrate a series of events which ultimately lead to host autophagy. NSP4 viroporin activity at the ER can activate the ER calcium sensor stromal interaction molecule 1 (STIM1), which triggers an activation of store-operated calcium entry (SOCE), which in turn facilitates Ca^{2+} influx through the plasma membrane (Hyser et al. 2013). This increase in intracellular Ca^{2+} activates the Ca^{2+}/calmodulin-dependent protein kinase kinase-β (CaMKK-β) to initiate autophagy (Anderson et al. 1999; Crawford et al. 2012). The autophagy membrane trafficking pathway is then hijacked by the virus to transport viral proteins from the ER to viroplasms for assembly of infectious virus (Crawford et al. 2012).

Intraviral and Host Interactions

Even before the discovery of its viroporin activity, NSP4 has been described to perform multiple functions through interacting with a number of viral and host factors, and the cytoplasmic region of NSP4 is an interaction hotspot. For instance, the NSP4 cytoplasmic CCD has been reported to be the binding site for the rotavirus spike protein VP4 (NSP4 aa112–148) (Au et al. 1993), host extracellular matrix (ECM) proteins laminin-β3 and fibronectin (NSP4 aa87–145) (Boshuizen et al. 2004), caveolin-1 (Cav-1) (NSP4 aa114–135) (Parr et al. 2006; Ball et al. 2013), and the integrin I domains (NSP4 aa114–130) (Seo et al. 2008). In addition, the flexible region of the NSP4 cytoplasmic tail interacts with VP6 to serve as an intracellular receptor for the viral double-layered particles (DLPs) (NSP4 aa161–175) (Au et al. 1989; Taylor et al. 1996) and can also bind microtubules (NSP4 aa120–175) (Xu et al. 2000). NSP4 can also bind the host calnexin via the two N-linked high-mannose oligosaccharide residues within the NSP4 H1 domain (Mirazimi et al. 1998). The putative binding sites for these interactions are summarized (Fig. 15.13).

While mechanistic and structural information on membrane insertion and oligomerization of the full-length NSP4 is still lacking, a topology model of NSP4 as a three-pass transmembrane protein has been proposed (Fig. 15.14). In addition, crystal structures have revealed that the NSP4 CCD from two different rotavirus strains can form a tetramer and a pentamer, respectively (Bowman et al. 2000; Chacko et al. 2011) and that the tetrameric NSP4 CCD, but not the pentameric form, can bind Ca^{2+} at its core. Later studies clarified that the oligomeric status of NSP4 CCD can be regulated by pH and Ca^{2+}; at neutral pH it forms a tetramer that binds Ca^{2+},

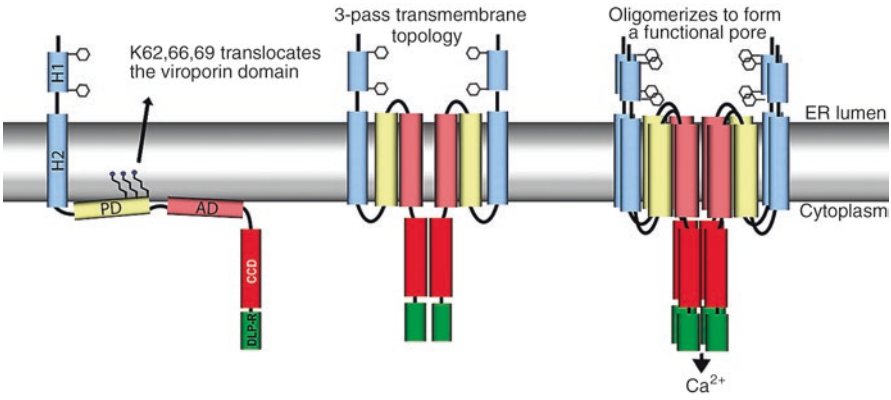

Fig. 15.14 Model of NSP4 as a three-pass transmembrane protein. Left: initial ER membrane insertion of NSP4 mediated by uncleaved signal sequence in H2 domain. Lysine residues interact with membrane phospholipid, promoting insertion of the viroporin domain (PD + AD) as an anti-parallel α-helical hairpin. Center: insertion of the viroporin domain generates a three-pass transmembrane topology. Right: NSP4 oligomerization around the amphipathic α-helix generates an aqueous pore for the passage of ER Ca^{2+} [adapted from Hyser et al. (2010)]

but at low pH it forms a pentamer that does not bind Ca^{2+} (Sastri et al. 2014). While the NSP4 CCD appears to be an interaction hotspot, it remains to be revealed how environmental cues may influence its binding conformations and affinity with interaction partners. For instance, the CCD may act as a cytoplasmic pH/Ca^{2+} sensor that alters the NSP4 oligomeric state and conformation to regulate its binding to a certain interaction partner, or Ca^{2+} may even act as a cofactor for binding.

Human Papillomavirus E5

Human papillomaviruses (HPVs) are double-stranded DNA viruses which are small, non-enveloped, and epitheliotropic. They are known to infect mucosal and cutaneous epithelia of the anogenital tract and the hands/feet regions. High-risk HPVs, mainly HPV-16 and HPV-18, are responsible for 70% of cervical cancers and precancerous cervical lesions. The HPV-16 E5 (16E5), a small hydrophobic oncoprotein, is a recent addition to the viroporin family as it exhibits ion channel activity in vitro (Wetherill et al. 2012). 16E5 is an 83-residue protein with three putative TMDs and an N-terminal luminal, C-terminal cytoplasmic topology (Krawczyk et al. 2010). 16E5 monomers oligomerize as homodimers or hexamers (Kell et al. 1994; Gieswein et al. 2003; Wetherill et al. 2012). 16E5 has roles in cellular transformation, mitogenic signaling, immune evasion, intracellular protein trafficking, and apoptosis [reviewed in Müller et al. (2015)].

Host Interactions

It has been reported that 16E5 forms a stable complex with the epidermal growth factor receptor (EGFR) in co-transfected cells (Hwang et al. 1995) and with the 16 K subunit of the vacuolar H^+-ATPase (V-ATPase) (Conrad et al. 1993), although the 16E5 binding site with the latter is under debate (Adam et al. 2000; Rodríguez et al. 2000). The C-terminal domain of 16E5 has been reported to bind the nuclear transport receptor karyopherin β3 (KNβ3) (Krawczyk et al. 2008) and the Ca^{2+}/phospholipid-/actin-binding protein calpactin I (Krawczyk et al. 2011). Other reported interaction targets of 16E5 include the gap junction protein connexin 43 (Oelze et al. 1995; Tomakidi et al. 2000), growth factor receptor ErbB4 (Chen et al. 2007), zinc transporter ZnT-1 (Lazarczyk et al. 2008), transmembrane channel-like proteins EVER1 and EVER2 (Lazarczyk et al. 2008), the putative ER ion channel A4 (Kotnik Halavaty et al. 2014), and the Golgi-resident transmembrane protein YIPF4 (Müller et al. 2015).

Interactions between 16E5 and host proteins have been implicated in the modulation of host defense. For instance, 16E5 can help HPV escape from immunesurveillance by downregulating expression of antigen-presenters at the host cell surface. 16E5 binds and retains MHC-I in the ER and Golgi to prevent its trafficking

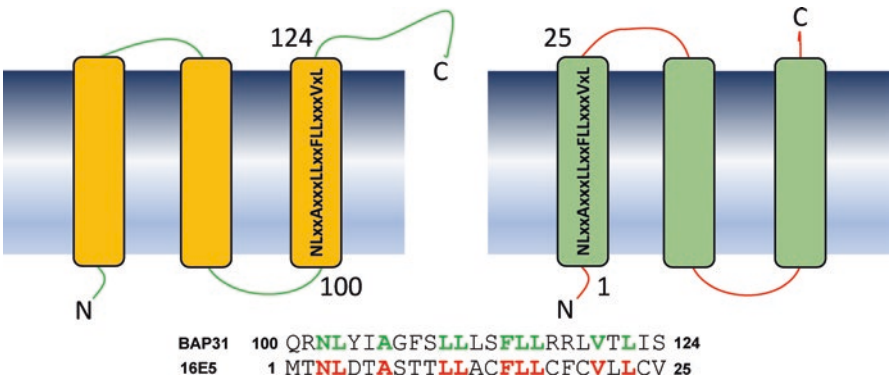

Fig. 15.15 E5 and BAP31 topology. Top, topology of E5 and Bap31, with TMDs indicated; TM3 in BAP31 and TM1 in 16E5 share a similar motif [adapted from Cortese et al. (2010)]. The alignment of 16E5 TM1 and Bap31 TM3 showing the ten-residue identity which may be involved in interactions among 16E5, MHC-I and/or other associated proteins. Identical residues are in bold

to the cell surface, in an interaction involving two leucine pairs in the first TMD (TM1) of 16E5 and the heavy chain of MHC-I (Ashrafi et al. 2005; Cortese et al. 2010; Ashrafi et al. 2006). The TM1 of 16E5 may also bind and cripple the function of the MHC-I chaperone, Bap31 (Ladasky et al. 2006; Regan and Laimins 2008; Cortese et al. 2010). Intriguingly, a motif consisting of ten identical residues between the 16E5 TM1 and Bap31 TM3 has been discovered (Fig. 15.15) and could represent a case of molecular piracy used by 16E5 to displace Bap31 from MHC-I. In addition, 16E5-mediated ER retention of MHC-I may also involve an interaction with the ER chaperone calnexin, since surface downregulation of MHC-I is not observed in calnexin-deficient cells (Gruener et al. 2007). In the same study, a tri-protein complex of 16E5, MHC-I, and calnexin could be obtained based on a coimmunoprecipitation assay. The 16E5-calnexin interaction also reduced CD1d surface levels by retaining it in the ER and subsequently redirecting it for proteasomal degradation (Miura et al. 2010).

BPV E5: A Case Study

E5 also plays an important role in cell tumorigenic transformation. One associated cellular target of the bovine papillomavirus BPV-1 E5 is the platelet-derived growth factor β receptor (PDGFβR), a transmembrane receptor tyrosine kinase (Fig. 15.16, left). Under normal circumstances, the ligand PDGF binds to the extracellular domain of its receptor to induce receptor dimerization, leading to the autophosphorylation of tyrosine residues at the receptor intracellular domain, activation of their intrinsic tyrosine kinase activity, and subsequent signal transduction resulting in mitogenesis (Fig. 15.16, middle). While the BPV-1 E5 is not a natural ligand of

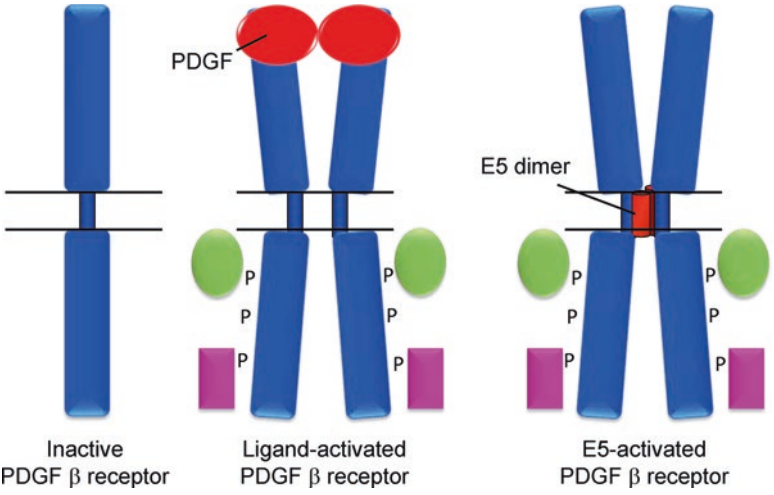

Fig. 15.16 Model for E5-PDGFβR interaction. *Left*, monomer of inactive PDGFβR; middle, PDGFβR activation by PDGF binding in the extracellular domain, leading to receptor dimerization, tyrosine phosphorylation in the intracellular domain, and recruitment of cellular signaling substrates (*green* and *purple*); right, PDGFβR activation by binding of a BPV-1 E5 dimer to the TMD of the receptor. Horizontal lines represent cell membrane, with the cytoplasm beneath [adapted from DiMaio and Petti (2013)]

PDGF*β*R, it can constitutively activate its receptor tyrosine kinase activity (Fig. 15.16, right) (Drummond-Barbosa et al. 1995) and is therefore an oncoprotein that can lead to host cell transformation.

Coimmunoprecipitation experiments have shown that the E5-PDGF*β*R interaction is stable and is important in E5-induced cell transformation (Petti and DiMaio 1992; Goldstein et al. 1992). Deletion of the extracellular ligand-binding domain of PDGF*β*R did not prevent its cooperation with E5, demonstrating that E5 activation of PDGF*β*R is ligand independent (Drummond-Barbosa et al. 1995). Also, mutagenesis and chimeric studies of PDGF*β*R have mapped the binding region to the TMD of the receptor (Cohen et al. 1993; Nappi et al. 2002). The interaction between E5 and PDGF*β*R is also highly specific, since cooperation was not observed between E5 and PDGFαR, a closely related receptor tyrosine kinase (Goldstein et al. 1994). Molecular dynamics experiments have proposed a model of the E5 dimer, where the Gln17 of monomer 1 and the Asp33 of monomer 2 are on the same face of the dimer that interacts with a molecule of PDGF*β*R (Surti et al. 1998). NMR studies of E5 peptides in detergent micelles also favor E5 dimerization for complex formation with PDGF*β*R through its TMD (King et al. 2011).

Concluding Remarks

The interplay between the host cell responses and viral offensive mechanisms yields the final outcome of an infection. For efficient viral replication, the virus must be resourceful in harnessing or disabling the host cellular machinery. In recent years, the role of viroporins as essential players in viral pathogenesis has been established. However, in addition to disrupting cellular ion homeostasis by their channel activity, viroporins also interact with host factors and coordinate with other viral proteins in structural roles. The structural features of these complexes remain poorly understood. Advances in this field will provide useful insights in the design of new antivirals.

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