Structure and Assembly

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Mutation of a putative Cholesterol Consensus Motif (CCM) of Influenza virus HA retards transport through the Golgi and affects association with membrane rafts

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The HA (hemagglutinin) of influenza viruses must be recruited to membrane rafts to perform its function in membrane fusion and virus budding. We have previously shown with a biophysical approach (FLIM-FRET) that deletion of the two raft-targeting features of HA, S-acylation at the cytoplasmic tail and the three hydrophobic amino acids VIL in the outer part of the transmembrane region (TMR) lead to reduced raft association [1,2]. In addition, exchange of VIL, but not of the S-acylation sites severely retards transport of HA through the Golgi [3].

Here we have further characterized the ill-defined signal in the TMR. A sequence comparison suggests that leucine (L) of VIL might be part of a cholesterol consensus motif (CCM) that is known to bind cholesterol to seven-TMR-receptors. The signal also comprises a lysine (K) and a tryptophan (W) on one and a tyrosine (Y) on another TMR-helix of the HA trimer. Interestingly, the CCM is conserved in group-2, but not present in group-1 HAs and for the latter mutation in the TMR has no effect on intracellular transport.

We created various mutants in the putative CCM and tested their effect on intracellular transport of HA by quantitatively analyzing its Golgi-localized processing using pulse-chase experiments and its association with rafts at the plasma membrane using FLIM-FRET. Exchange of L was delaying HA´s transport to a similar extent as exchange of the whole VIL motif whereas exchange of I, which is not part of the CCM, had no effect on transport. Additionally, exchange of W, K and Y also retards or even abolishes transport of HA to the plasma membrane. FLIM-FRET revealed that already W had an effect on clustering of HA with rafts, which was increased by additionally mutating the neighboring L. Doublemutation of K and Y also showed a similar effect than L and W.

The results are consistent with the model that HA contains a CCM located at the boundary between its ectodomain and TMR. Association with cholesterol might facilitate transport of HA along the exocytic pathway, where the cholesterol concentration steadily increases, and association with rafts at the plasma membrane, which contain cholesterol as an essential element.

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Oligomerization of the hepatitis B virus small envelope protein S

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The small envelope protein S of the hepatitis B virus is cotranslationally inserted into the ER membrane and traverses the lipid bilayer probably four times with transmembrane domains TM1 – TM4. The S protein is incorporated into the envelope of virions and, in addition, forms subviral lipoprotein particles (SVP) of 20 nm diameter which appear in the lumen of the secretory compartment and are released from S expressing cells in large quantities. The biogenesis of SVP is not well understood. Recently, we showed that the authentic sequence of TM1 and TM2 are important for SVP formation. We now generated fusion constructs of S and fluorescent proteins (YFP and BFP) and analyzed their interaction by fluorescence resonance energy transfer (FRET) detection in cotransfected Huh7 cells in a FACS machine. Chimera containing only TM2 at the C terminus of YFP and BFP, respectively, generated a high FRET signal suggesting that TM2 directs the formation of homooligomers in the absence of other parts of S. Substitution of TM2 alone or in combination with TM1 by foreign but functionally homologous TMs in the S background showed no reduction of the FRET signal even when TM3/4 were additionally deleted. This indicates further parts of the protein are involved in the oligomerization process independently of the transmembrane domains. Surprisingly, a C65S mutation in the cytoplasmic loop of S led to a drastic reduction of the FRET signal. This is consistent with older observations that all 4 cysteines in this loop were crucial for SVP formation. Further experiments to investigate the role of these cysteines in SVP biogenesis are under way.

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Retention and intracellular dynamics of the Pestivirus Envelope Protein Erns

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Pestiviruses are positive strand RNA viruses of the family *Flaviviridae*. The genus *Pestivirus* contains viruses that infect different farm animals including ruminants, among them the prototype virus bovine viral diarrhea virus (BVDV). BVDV has three envelope proteins, E^{rns}, E1 and E2. E1 and E2 are only found on the viral particle, whereas E^{rns} is part of the viral particle but is also secreted from infected cells,

and found in considerable amounts in the blood of infected animals. BVDV particle formation occurs intracellularly and the virus buds into the ER. It has been shown that E^{rns} is retained within the cell and not expressed on the cell surface. I analyzed the subcellular localization of E^{rns}, to characterize its retention site in infected cells compared to cells expressing the protein ectopically. Furthermore, the dynamics of E^{rns} retention were analyzed in comparison to E1 and E2 using live cell imaging and fusion proteins with fluorescent proteins, as well as immunofluorescence microscopy. Interestingly the intracellular mobility of ectopically expressed E^{rns}, as well as its localization differed from that observed for E1 and E2. Both E1 and E2 are bound to membranes by classical transmembrane domains, whereas E^{rns} contains a c-terminal amphipathic helix that anchors it in the membrane. Different mutants of E^{rns} were used to better characterize the role the special membrane anchor of E^{rns} plays in the intracellular mobility observed. A better understanding of the dynamics of E^{rns} retention might help gain insights into the equilibrium between secreted and retained E^{rns} and thus advance our knowledge of its role in viral pathogenesis.

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The highly conserved Serine 40 of HIV-1 p6 regulates polyubiquitination and immunogenicity of Gag

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The HIV-1 p6 Gag protein contains two late assembly (L-) domains that recruit members of the ESCRT pathway to mediate membrane fission between the nascent virion and the cell membrane. In addition, the highly conserved serine in position 40 regulates capsid processing, without affecting ESCRT recruitment and virus release, indicating an L-domain independent late function of p6. Recently, it has been demonstrated by others that the mutation of Ser-40 to phenylalanine (S40F) induces the formation of Gag-containing filopodia-like structures. As the cellular factor syntenin has been implicated in this process and is known to interact with mono-ubiquitin as well as K48- and K63-linked polyubiquitin, we wanted to investigate the Gag ubiquitination of the S40F mutant. Until now it has been established that enhanced membrane association of Gag, caused by L-domain mutations, augments the polyubiquitination of Gag by ubiquitin ligases localized in the membrane compartment.

Here we show that the S40F mutation leads to an increased membrane association and ubiquitination of Gag which is predominantly constituted of K48-linked polyubiquitin chains. Consequently, the S40F mutant augments the entry of Gag into the ubiquitin proteasome system and causes enhanced MHC-I antigen presentation of Gag derived epitopes and thus Gag-mediated T-cell activation. Additionally, Ser-40 mediated ubiquitinated Gag species participate in the assembly process and are efficiently incorporated into virus particles, where they might disturb Gag processing (e.g. accumulation of CA p25) and subsequently reduce the infectivity of progeny virions. Thus, the increased Gag ubiquitination regulated by a region in p6 other than L-domains does not correlate with virus release and most likely does not play a role in the budding process. However, the elevated polyubiquitination of Gag might enhance the immunogenicity of Gag that parallels with a loss in Gag processing and infectivity.

Structure and Assembly

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A Concerted Function of Arginine Residues in the Prototype Foamy Virus Gag C-terminus Mediates Viral and Cellular RNA Encapsidation

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Foamy viruses (FVs) are a special type of retroviruses that combine replication characteristics of both orthoretroviruses and hepadnaviruses. One of several unique features of the FV capsid protein Gag is the absence of Cys-His motifs, which in orthoretroviruses are irreplaceable for multitude functions including viral RNA recognition and packaging. Instead, FV Gag contains glycine-arginine-rich (GR) sequences at its C-terminus. In case of prototype FV (PFV) these are grouped in three boxes, which have been shown to play essential functions in genome reverse transcription, virion infectivity and particle morphogenesis. Additional functions for RNA packaging and Pol encapsidation were suggested, although the contributions of individual boxes are controversially discussed. Here we show that the concurrent deletion of all three PFV Gag GR boxes or the substitution of 23 arginine residues residing in the C-terminal GR box region by alanine abolished both viral and cellular RNA encapsidation (>3,000-fold reduced), albeit having only a moderate effect on particle release (3 to 10-fold). Consequently, those mutants also lacked Pol and were non-infectious. In contrast, deletion of individual GR boxes had only minor effects (2 to 4-fold) on viral and cellular RNA encapsidation over a wide range of cellular Gag to viral genome ratios examined. Taken together, our data provides the first description of cellular RNA encapsidation into FV particles and characterization of Gag mutants devoid of both viral and cellular RNA. Our results suggest that the concerted action of C-terminal clustered positively charged residues is the main PFV Gag determinant for vgRNA encapsidation. Furthermore, this indicates that non-primate FVs, which lack GR boxes, might use a similar mechanism of genome packaging.