SnapShot: Enveloped Virus Entry

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1 Virus binding

| Virus                 | Glycoprotein (GP) | SARS CoV (Severe acute respiratory syndrome coronavirus) | Influenza virus | HIV (human immunodeficiency virus) |
|-----------------------|-------------------|----------------------------------------------------------|-----------------|-----------------------------------|
| VACV (Vaccinia virus) |                   |                                                          |                 |                                   |
| EBOV (Ebola virus)    |                   |                                                          |                 |                                   |
| SARS CoV-2            | Spike protein (S)  |                                                           |                 |                                   |
| RSV (Respiratory syncytial virus) | Fusion glycoprotein (F) |                                                   |                 |                                   |
| Influenza virus       | Hemagglutinin (HA) |                                                          |                 |                                   |
| HIV                   | Env glycoprotein (gp130) |                                                       |                 |                                   |

2 Internalization

| Virus                    | Clathrin-mediated endocytosis | SARS CoV, Influenza, HIV | SARS CoV, Influenza, HIV |
|--------------------------|--------------------------------|--------------------------|--------------------------|
| EBOV, RSV, VACV, HIV     | Macropinocytosis               | Cell-surface fusion      |                          |

3A Trafficking

A. Receptor recycling
B. Maturation
C. Fusion with lysosomes

3B Exposure of fusogenic peptides

Table. Viruses with Class I Fusion Proteins

| Virus family (representative) | Viral fusogen | Enzymatic processing (location) | Fusion pH |
|-------------------------------|---------------|---------------------------------|-----------|
| Retroviridae (HIV)            | Env glycoprotein (gp120) | furin (Golgi)                   | neutral   |
| Paramyxoviridae (RSV)         | Glycoprotein (GP) | furin (Golgi); cathepsins, i.e., catB, catL (endosome) | acid |
| Flaviviridae (EBOV)           | Spike glycoprotein (S) | furin (Golgi); cathepsins, i.e., catB, catL (endosome) | acid |
| Coronaviridae (SARS CoV-2)    | Spike glycoprotein (S) | furin (Golgi); cathepsins, i.e., catB, catL (endosome) | acid |
| Orthomyxoviridae (Influenza)  | Hemagglutinin (HA) | furin (Golgi); TMPRSS2 (extracellular), catB, catL, catL (endosome) | acid |
| Orthomyxoviridae (Influenza)  | Hemagglutinin (HA) | furin (Golgi); cathepsins, i.e., catB, catL (endosome) | acid |

4 Insertion

A. Extended intermediate
B. Collapse of intermediate
C. Hemifusion
D. Fusion pore

5 Membrane fusion and genome release

A. Extended intermediate
B. Collapse of intermediate
C. Hemifusion
D. Fusion pore

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Despite their many differences and various sites of fusion, the common principles of enveloped virus entry and the membrane fusion step are largely conserved. Here, we describe the sequence of events leading to the entry of a subset of representative enveloped viruses.

(1) Virus Binding

Virus receptors can be divided into two categories: attachment factors and entry receptors (Marsh and Helenius, 2006). Attachment factors concentrate viruses on the cell surface, while entry receptors are bona fide signaling proteins that initiate endocytosis. Chondroitin- and heparan-sulfate proteoglycans (CSPGs and HSPGs, respectively) are negatively charged polysaccharides that act as typical attachment factors for most enveloped viruses. Through low-affinity, high-avidity interactions, these glycosaminoglycans promote virus binding and their subsequent engagement with entry receptors. Entry receptors must meet several criteria: they must contact the virus, activate signaling cascades which trigger endocytosis, and be essential for virus internalization. Most viruses use multiple entry receptors, which can act to facilitate structural changes in viral proteins to allow co-receptor binding, extend infection to different cell types or hosts, or simultaneously downregulate host immune responses.

(2) Internalization

While some enveloped viruses are capable of fusing at the cell surface, the majority enter cells by endocytosis (Meric et al., 2010). Endocytic entry provides many advantages to viruses. First, while in an endosome, viruses obviate the need to encode accessory proteins for passage through cortical actin or for microtubule-based transport. Second, no evidence of infection is left on the cell surface—slowing immune responses. Third, for viruses that use constitutive endocytic programs, there is no need to actively trigger uptake. Fourth, endosomes provide cues for virus priming and fusion (see Lee et al., 2008; Millet and Whittaker, 2015). In addition, cells present multiple endocytic pathways. Like the viruses attempting to gain entry, these pathways differ in size and shape (from 100 nm clathrin-coated pits to 10 µm macropinosomes), allowing for endocytosis of small (influenza, 100 nm) and large (vaccinia, 370 nm) viruses individually or as multiples. Finally, each pathway can be triggered by different receptors—defined by a subset of critical cell factors (actin, dynamin, cholesterol, etc.)—for the formation, closure, and scission of primary endocytic vacuoles from the plasma membrane.

(3) Trafficking

Regardless of the uptake mechanism, internalized viruses are delivered into the endosomal system. The endosomal pathway is composed of several endosome classes: early endosomes (EEs), late endosomes (LEs), recycling endosomes (REs), and lysosomes (Lys). These organelles are differentiated by their Rab GTPase and phosphoinositide constituents, which act to coordinate endosome maturation in preparation for delivery of the incoming cargo to Lys for degradation. Maturation involves microtubule-based movement toward the cell center, a switch in Rab [Rab5 (EE) → Rab7 (LE)] and phosphoinositide [PI(3,5)P2 (EE) → PI(3,5,7)P3, (LE)] composition, a drop in pH [6.5 (EE) → 4.0 (Lys)], and activation endosomal proteases. Enveloped viruses take advantage of endosomal maturation to facilitate their pericentric movement, capsid/core priming, and proton- and/or protease-dependent activation of their fusion machinery. For example, the glycoprotein (GP) trimer of Ebola serves as a prototypical fusion protein, composed of a receptor-binding unit and a fusion subunit (Lee et al., 2008). The two subunits arise from furin-mediated cleavage of the precursor polypeptide by viral entry and remain associated via disulfide bonds and noncovalent interactions. The fusion subunit of the GP is only liberated upon cleavage of the two subunits by cathepsins. With its fusogenic peptides exposed, the viral fusion can insert into permissive host endomembranes.

(4) Insertion

The triggering of membrane fusion, preceded by conformational changes of the viral fusion protein, is initiated by the insertion of the viral fusion peptide(s) into the inner leaflet of the host membrane. Peptide insertion can be Ca2+-dependent, enhanced by cholesterol, and presumably coincident with displacement of glycoprotein coats (e.g., lysosome-associated membrane glycoproteins [LAMPS]).

(5) Membrane Fusion and Genome Release

(A) Peptide insertion results in contact between the bilayers, termed the extended intermediate, but there remains a gap between viral and host membranes and an energetic barrier to fusion (Harrison, 2008). (B) To lower this barrier, the inserted peptide increases the order of the host membrane and the large structural rearrangements to the viral envelope cause a collapse of the extended intermediate, decreasing the hydration repulsion of the apposing membranes. The collapse results in an extended, highly stressed bulge in the bilayer primed for hemifusion (Chernomordik and Kozlov, 2008). High membrane curvature is opposed by hydrostatic tension on membranes. Therefore, fusion also requires the extrusion of osmolytes from the endosome to relieve hydrostatic pressure, mediated in part by lipid-gated cation channels (Freeman et al., 2020; Ou et al., 2020; Sakurai et al., 2015). (C) Local tension that supports the curvature and fusion is contributed by the viral fusogen itself and possibly other sources. (D) A universal feature of viral fusogens is that their two membrane-associated elements (the fusion peptide or loop and the transmembrane anchor) come together to facilitate the formation of a fusion pore, through which the viral genome is released into the cytosol (Kilcher and Mercer, 2015).

ABBREVIATIONS

GBPs, GAG-binding proteins; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrim; TIM, T cell immunoglobulin and mucin receptor; V-ATPase, vacular-type H+–ATPase.

REFERENCES

Benton, D.J., Gamblin, S.J., Rosenthal, P.B., and Skehel, J.J. (2020). Structural Transitions in Influenza Haemagglutinin at Membrane Fusion pH. Nature 583, 150–153.
Chernomordik, L.V., and Kozlov, M.M. (2008). Mechanics of membrane perforation. Nat. Struct. Mol. Biol. 15, 675–683.
Freeman, S.A., Uderhardt, S., Saric, A., Collins, R.F., Buckley, C.M., Mylvaganam, S., Boroumand, P., Plumb, J., Germain, R.N., Ren, D., and Grinstein, S. (2020). Lipid-gated monovalent ion fluxes regulate endocytic traffic and support immune surveillance. Science 367, 301–305.
Harrison, S.C. (2008). Viral membrane fusion. Nat. Struct. Mol. Biol. 15, 690–698.
Kilcher, S., and Mercer, J. (2015). DNA virus uncoating. Virology 479–480, 578–590.
Lee, J.E., Fusco, M.L., Hessell, A.J., Oswald, W.B., Burton, D.R., and Saphire, E.O. (2008). Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature 454, 177–182.
Marsh, M., and Helenius, A. (2006). Virus entry: open sesame. Cell 124, 729–740.
Mercer, J., Schelhaas, M., and Helenius, A. (2010). Virus entry by endocytosis. Annu. Rev. Biochem. 79, 803–833.
Millet, J.K., and Whittaker, G.R. (2015). Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. Virus Res. 202, 120–134.
Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., et al. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat. Commun. 11, 1820.
Sakurai, Y., Kolokoltsov, A.A., Chen, C.C., Tidwell, M.W., Bauta, W.E., Klugbauer, N., Grimm, C., Wahl-Schott, C., Biel, M., and Davey, R.A. (2015). Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. Science 347, 995–998.