Molecular & Medical Virology

Virus Entry and Uncoating

22.4.2026

Dr. rer. nat. Nicola Frericks
Department of Molecular and Medical Virology
Medical Faculty
Ruhr-University Bochum

Yup that‘s me and you probably wondering how I ended up in this situation…

B.Sc. Molecular Biology (Westfälische Hochschule Recklinghausen)

M.Sc. Medical Biology (University Duisburg-Essen)

Dr.rer.nat in the field of Virology (Medical School Hannover, Twincore)

Since April 2023: Postdoctoral Research Assistant

Coming back to work…

Viruses have a single mission:

…transport the viral genome from an infected cell to a non-infected host cell where it can replicate and produce infectious progeny virus.

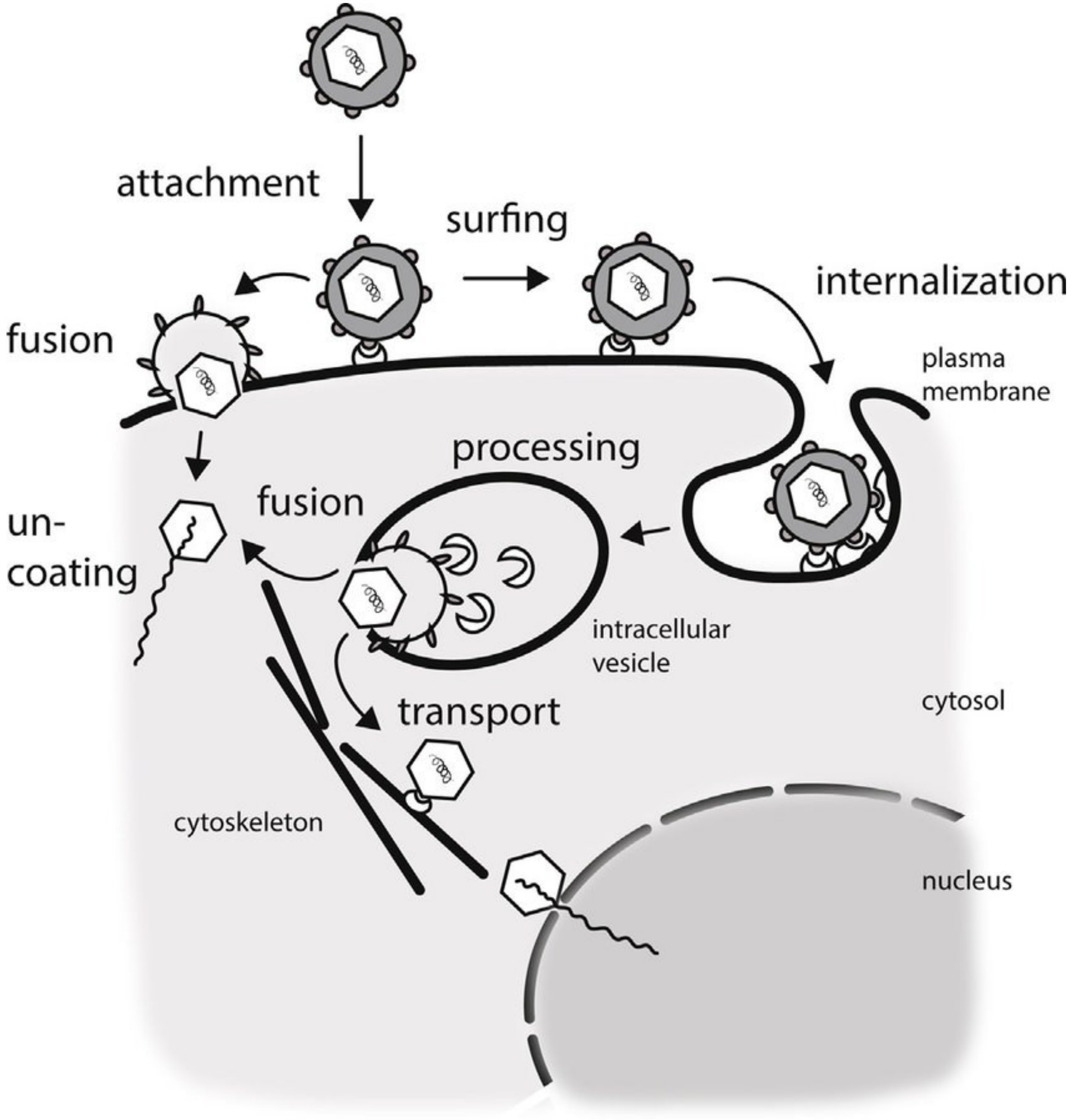
Problems:

- Viral particles are too large to passively diffuse across the plasma membrane
- The viral genome is encapsidated in a stable coat that shields the nucleic acid from degradation
- Viruses are obligate intracellular parasites and have only limited independent functions

- **Trojan horse strategy:** virus entry solely relies on hijacking normal cellular processes like endocytosis, membrane fusion, vesicular trafficking and transport into the nucleus

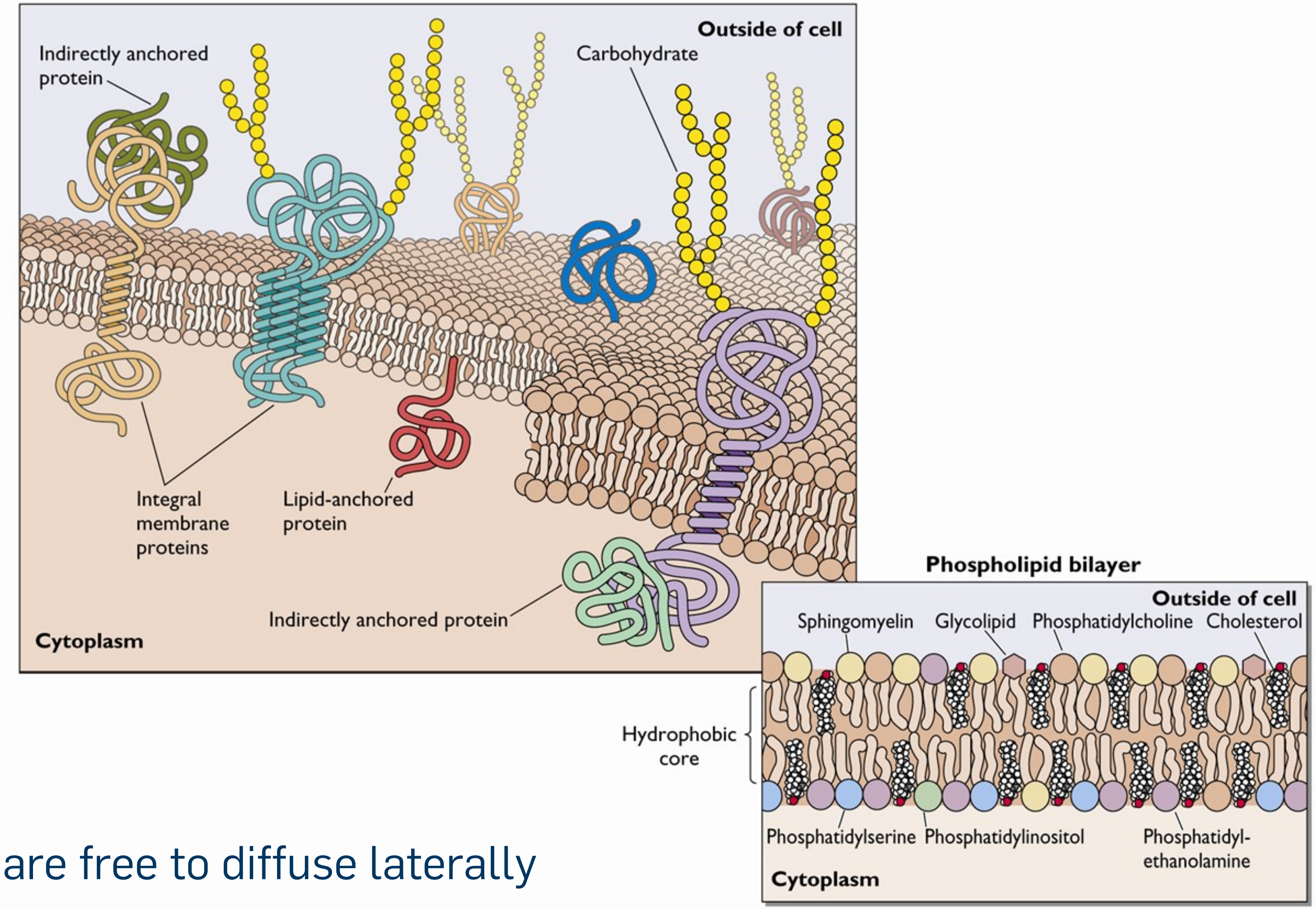
Basic steps of virus entry

1. **Attachment**
2. **Receptor binding**
3. **Internalisation/Fusion**
4. **Penetration**
5. **Uncoating**
6. **Intracytosolic transport of the genome to the site of viral replication**
7. **Nuclear import**



The barriers

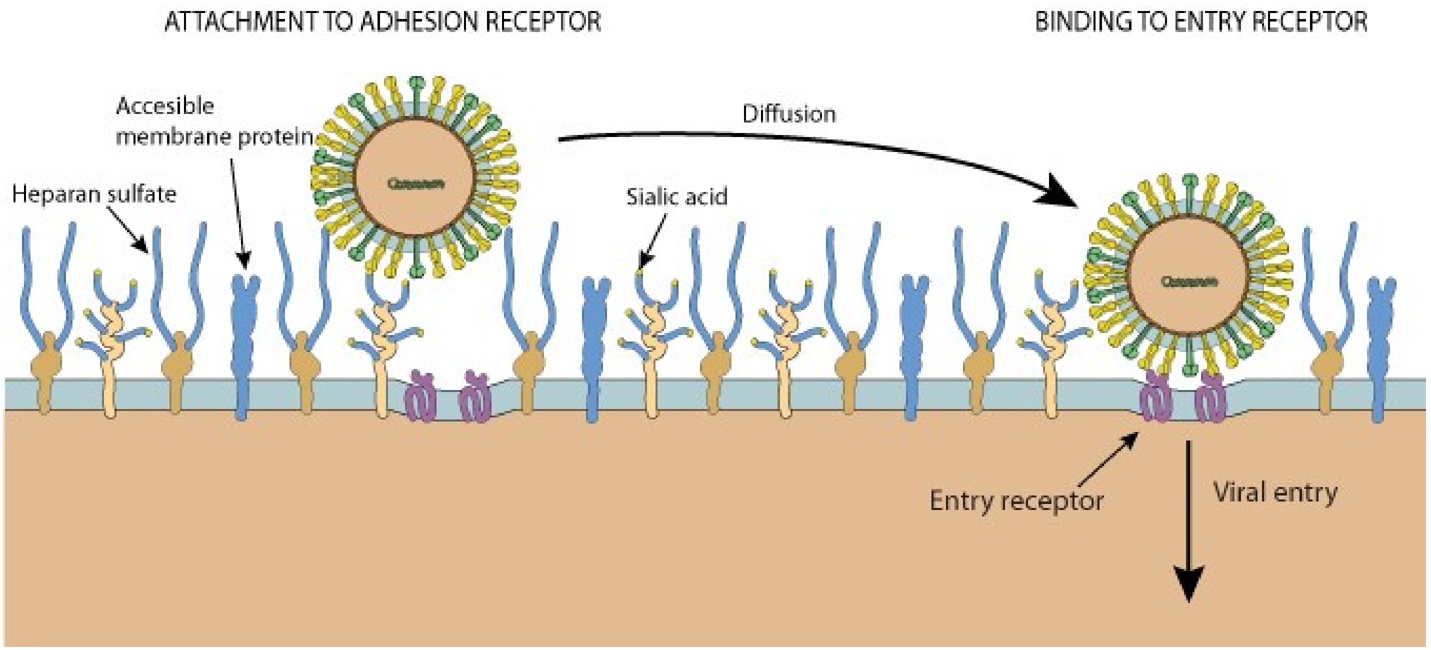
- **Glycocalyx**
  - Layer of glycoconjugates that covers the external surface of the cell
  - Glycoproteins, glycolipids, proteoglycans



- **Plasma membrane**
  - Phospholipid bilayer
  - Both phospholipids and proteins are free to diffuse laterally
  - Microdomains enriched in cholesterol and the sphingolipids = lipid rafts

Attachment

- **Viruses can only infect cells to which they can bind**
- **Attachment factors help to concentrate particles on the cell surface**
  - Not specific, irreversible
  - Enhance entry and infection
  - They do <u>NOT</u> actively promote entry or mediate host signaling



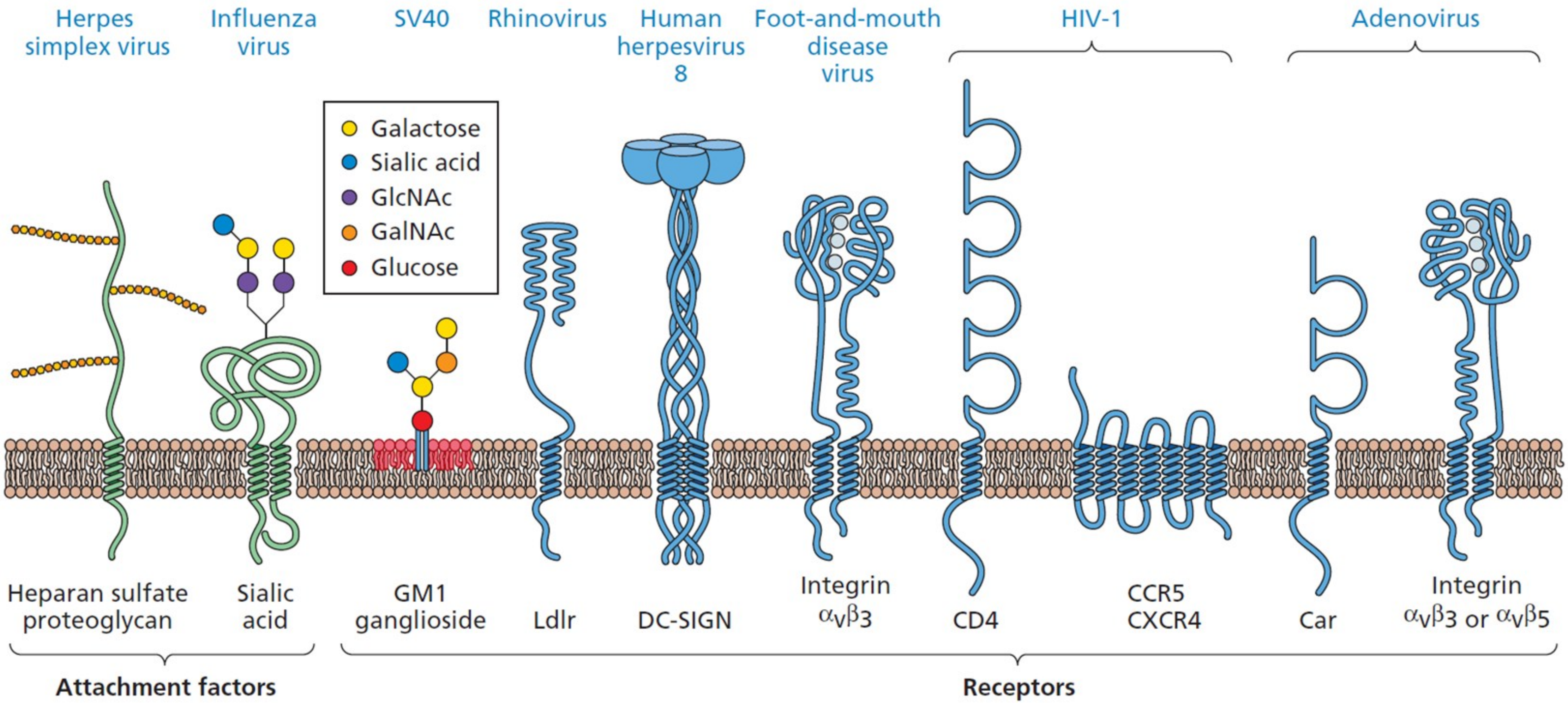
Receptor binding

- **Virus receptors are cell surface molecules that bind incoming virus and…**
  - Induce conformational changes on the viral surface

    → priming, association with other receptors, membrane fusion and penetration
  - Transmit signals trough the plasma membrane → virus uptake
  - Guide bound particles into a variety of endocytic pathways
- **Virus-receptor interaction is very specific and mostly direct (only few exceptions!)**
- **Affinity for a single virus is low**
  - Presence of multiple receptor-binding sites on the virion = avidity (strength of an interaction) is very high

    = Irreversible binding
  - Formation of microdomains which are rich in receptors and different in membrane properties

Some examples...



Molecular & Medical Virology

Some exa

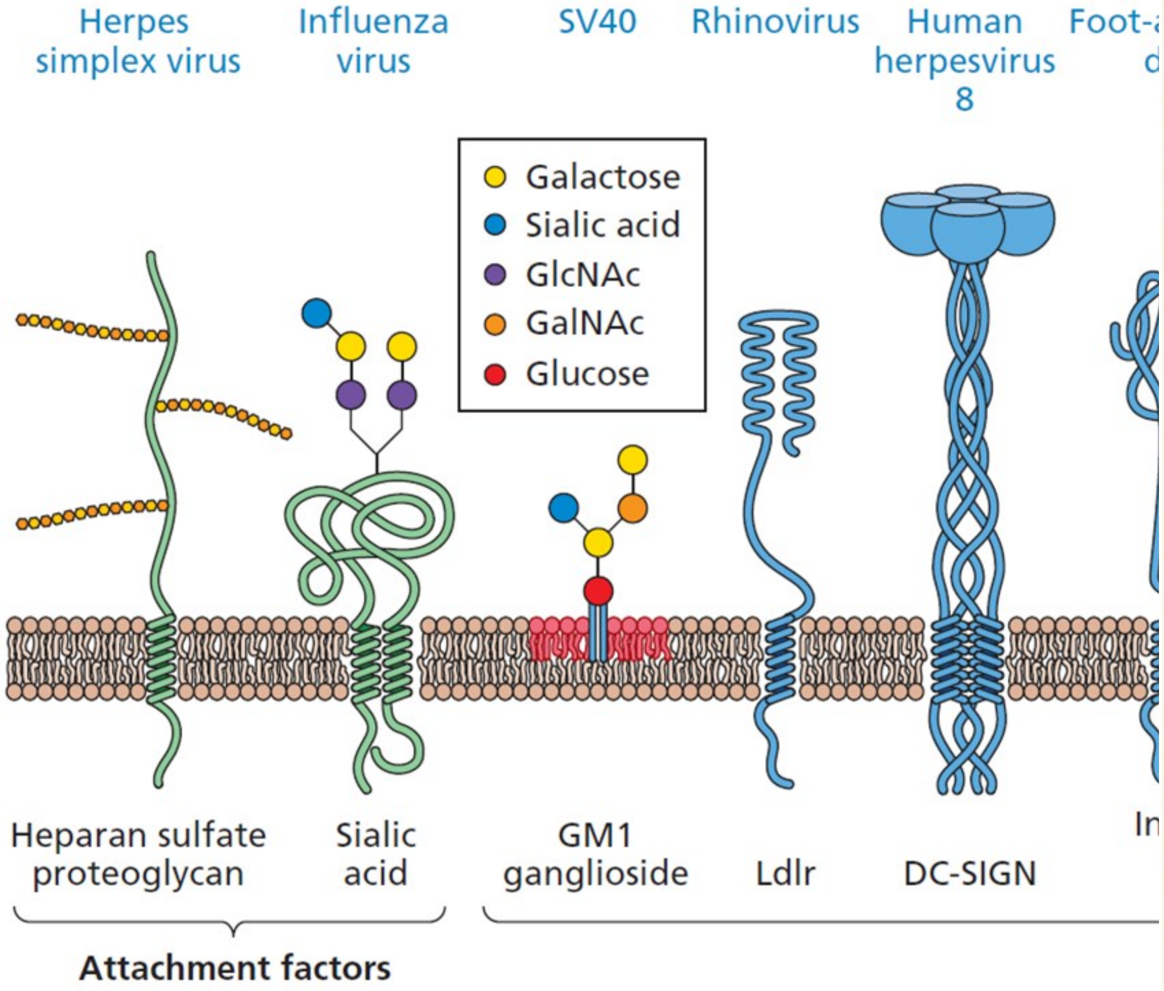


TABLE 4.1 Receptor Proteins for Some Viruses

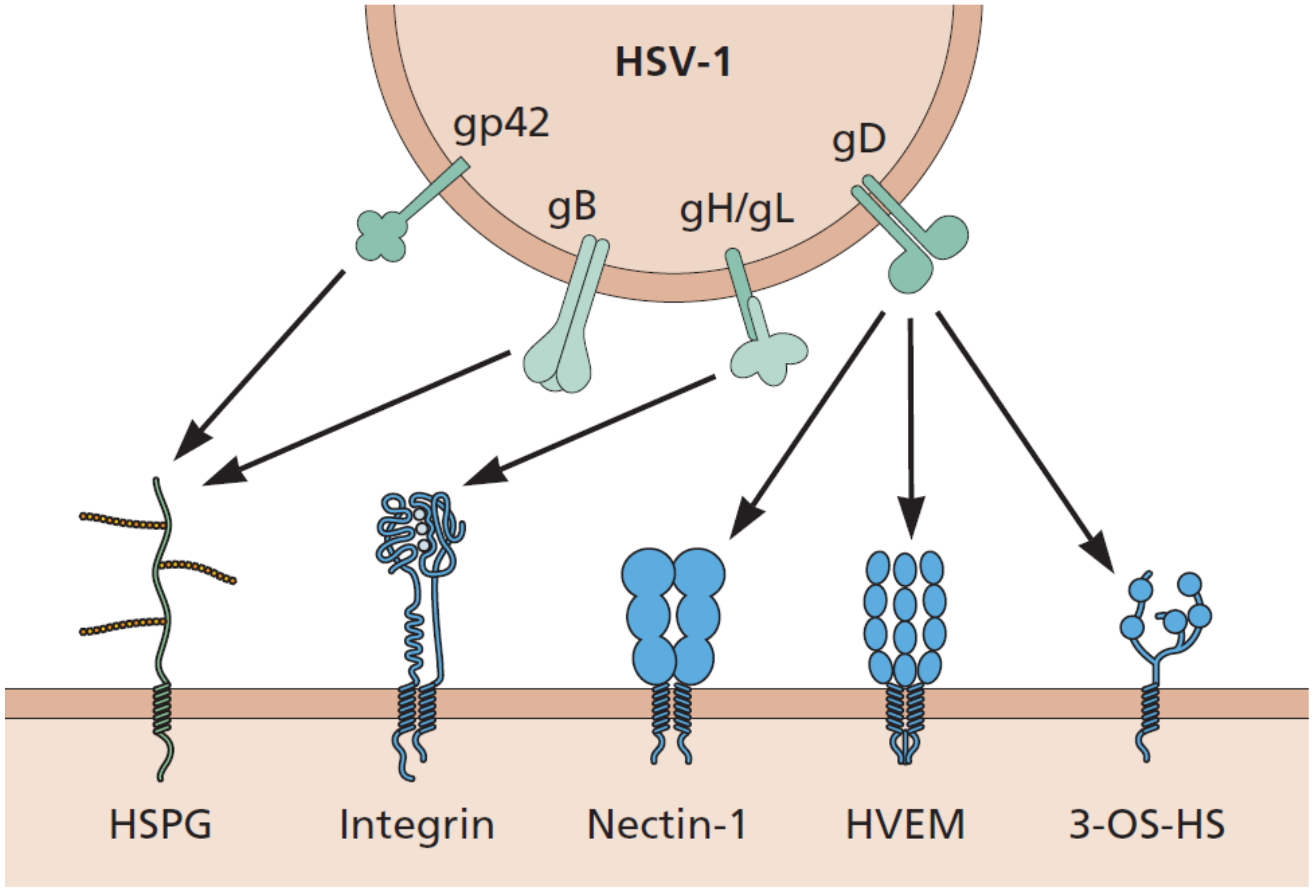
Virus	Family	Receptor	Function	References
G-protein-coupled receptors				
HIV	*Retroviridae*	CXCR4,CCR3,CCR2b,CCR8 CCR5	Chemokine receptors	2,32,47,163
HIV/SIV	*Retroviridae*	CCR5, Bonzo/STRL-33/ TYMSTR, BOB/GPR15, GPR1	Chemokine receptors	3,56,99
Proteins with multiple membrane-spanning domains				
GALV/FeLV-B/SSAV	*Retroviridae*	PiT-1	Phosphate transport	137,196
MLV-E	*Retroviridae*	MCAT-1	Cationic amino acid transport	1
MLV-A	*Retroviridae*	PiT-2	Phosphate transport	125,205
MLV-X/MLV-P	*Retroviridae*	XPR1/Rmc1/SYG1	Transporter	8,195
HCV	*Flaviviridae*	CD81	Tetraspanin membrane protein	147
Immunoglobulin-related proteins				
Poliovirus	*Picornaviridae*	PVR (CD155)	Adhesion receptor	121
PRV/BHV-1	*Herpesviridae*	PVR (CD155)	Adhesion receptor	67
HSV-1/HSV-2/PRV	*Herpesviridae*	Prr2/HveB/nectin-2	Adhesion	55
HSV-/HSV-2/	*Herpesviridae*	Prr1/HveC/nectin-1	Adhesion	67
Coxsackie B	*Picornaviridae*	CAR	Homotypic cell interaction	9,198
Ad-2/Ad-5	*Adenoviridae*	CAR	Homotypic cell interaction	10,198
MHV-A59	*Coronaviridae*	MHVR/Bgp1 (a)	Biliary glycoprotein	49
Human rhinoviruses (type B, and A major group)	*Picornaviridae*	ICAM-1	Cell adhesion/signaling	71,188
HIV/SIV	*Retroviridae*	CD4	T-cell signaling	106
HHV-7	*Herpesviridae*	CD4	T-cell signaling	104
Low-density lipoprotein receptor–related proteins				
Rous Sarcoma virus (type A)	*Retroviridae*	LDLR	Lipoprotein receptor	7
Human rhinoviruses (type A, minor group)	*Picornaviridae*	LDLR/α 2MR/LRP	Lipoprotein receptors	80
Integrins				
Adenovirus	*Adenoviridae*	$\alpha v\beta 3$	Vitronectin binding	213
Coxsackie A9	*Picornaviridae*	$\alpha v\beta 3$	Vitronectin binding	159
Adenovirus	*Adenoviridae*	$\alpha v\beta 5$	Vitronectin binding	214
Echoviruses-1/-8	*Picornaviridae*	$\alpha 2\beta 1$	Collagen/laminin binding	12
Foot-and-mouth-disease virus	*Picornaviridae*	$\alpha 2\beta 1$, $\alpha v\beta 3$, $\alpha v\beta 6$	Vitronectin binding	14,84
Hantaan virus	*Bunyaviridae*	$\alpha 3$ integrins		65
Rotavirus	*Reoviridae*	$\alpha 4\beta 1$, $\alpha v\beta 3$, $\alpha 2\beta 1$		78
Cytomegalovirus	*Herpesviridae*	$\alpha v\beta 3$, $\alpha 2\beta 1$, $\alpha 6\beta 1$		58
Tumor necrosis factor receptor–related proteins				
ALV-B/D/E	*Retroviridae*	TVB	Apoptosis-inducing receptor	17
Herpes simplex virus 1	*Herpesviridae*	HveA	LIGHT receptor	26,115
Small consensus repeat–containing proteins				
Epstein-Barr virus	*Herpesviridae*	CR2	C3d/C3dg/iC3b binding	59,60
Measles	*Paramyxoviridae*	CD46	Complement inhibition	48
Echoviruses	*Picornaviridae*	CD55	Complement inhibition	9
Coxsackie B-1/-3/-5	*Picornaviridae*	CD55	Complement inhibition	11,173
Miscellaneous				
Coronavirus-229E/TGEV	*Coronaviridae*	Aminopeptidase-*N*	Metalloproteinase	11,217
LCMV/Lassa fever virus	*Arenaviridae*	α-Dystroglycan	Laminin/agrin binding	24

Many viruses can use multiple receptors…

Example: Herpes Simplex Virus 1

- Glycoproteins gB and gp42 bind to heperan sulfate proteoglycan
  = attachment
- gH/gL interact with Integrin
- gD can bind to any of the 3 receptors: HVEM, Nectin-1 or 3-O-sulfated heparan sulfate

→ Flexibility enables infection of a variety of cell types

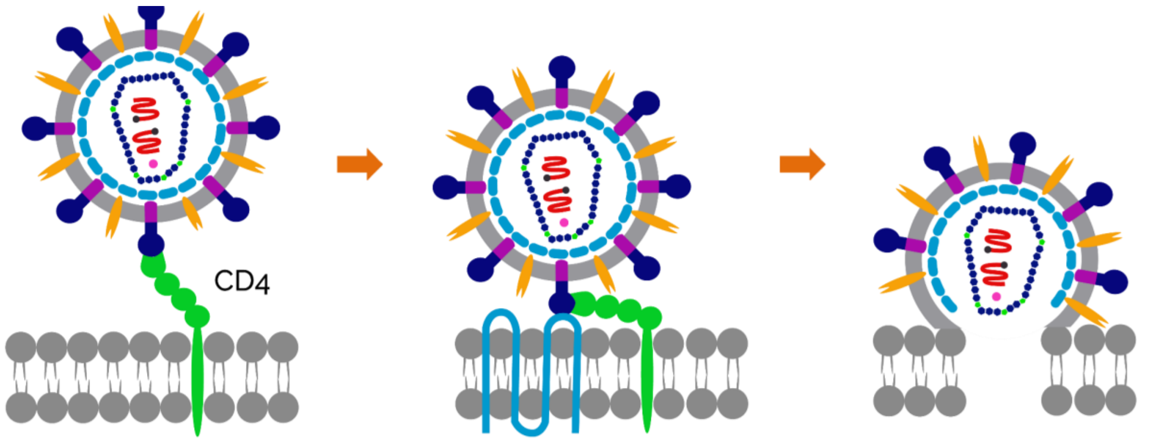


Stay in line...

Virus entry is a highly controlled process:

- First interaction = receptor
- Subsequent interactions = co-receptors

Example: Human Immunodeficiency Virus



HIV docks to the CD4 receptor on the host cell via glycoprotein gp120

Conformation changes in gp120 permit binding to co-receptor chemokines e.g. CCR5 or CXCR4

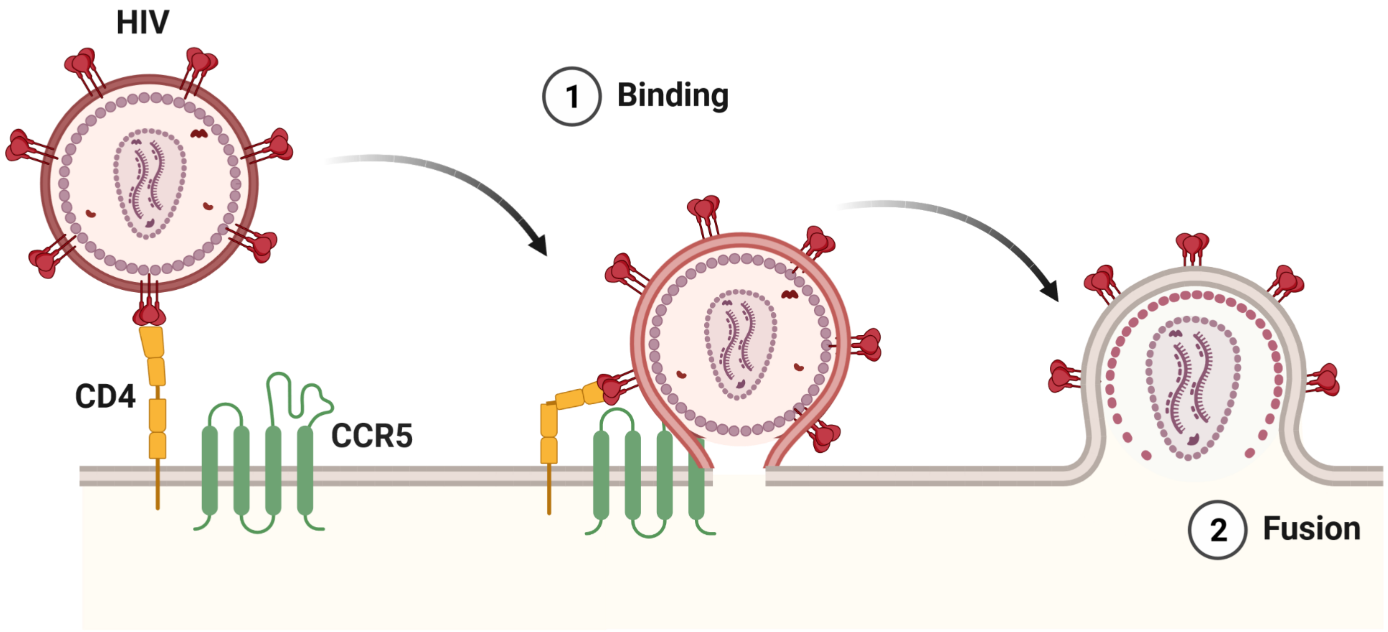
Further changes to the gp41 transmembrane protein allow for fusion with the cell membrane

Stay in line...

Virus entry is a highly controlled process:

- Conformational change is required to induce fusion

Example: Human Immunodeficiency Virus

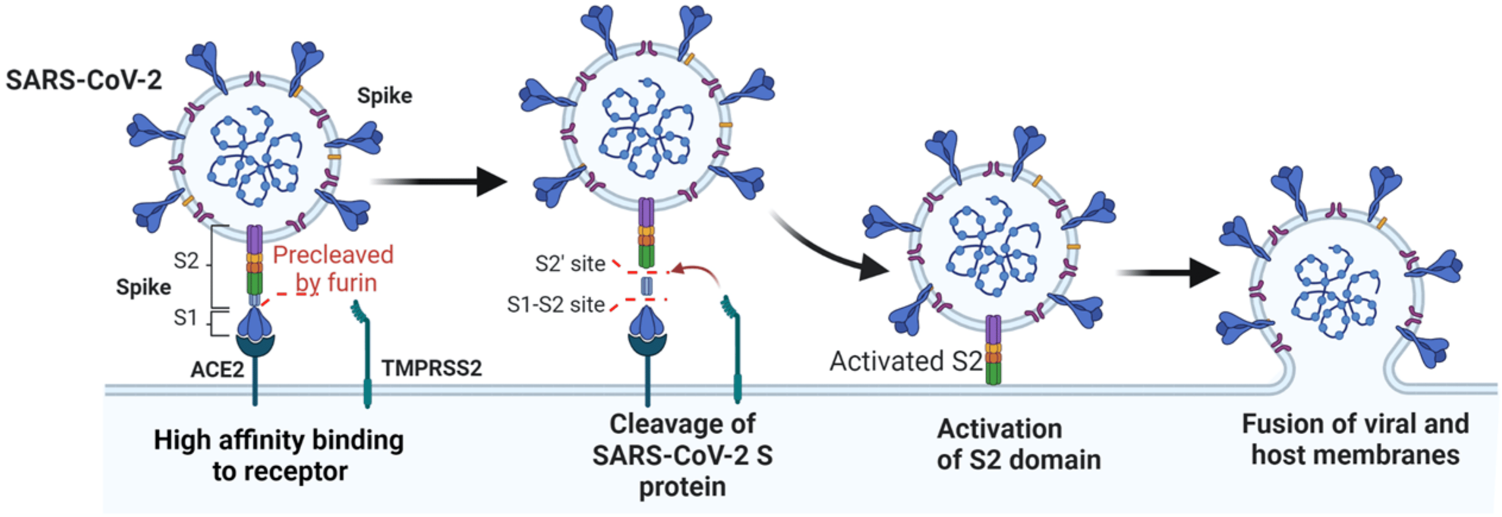


Stay in line...

Virus entry is a highly controlled process:

- Protease-dependent cleavage is required to induce fusion

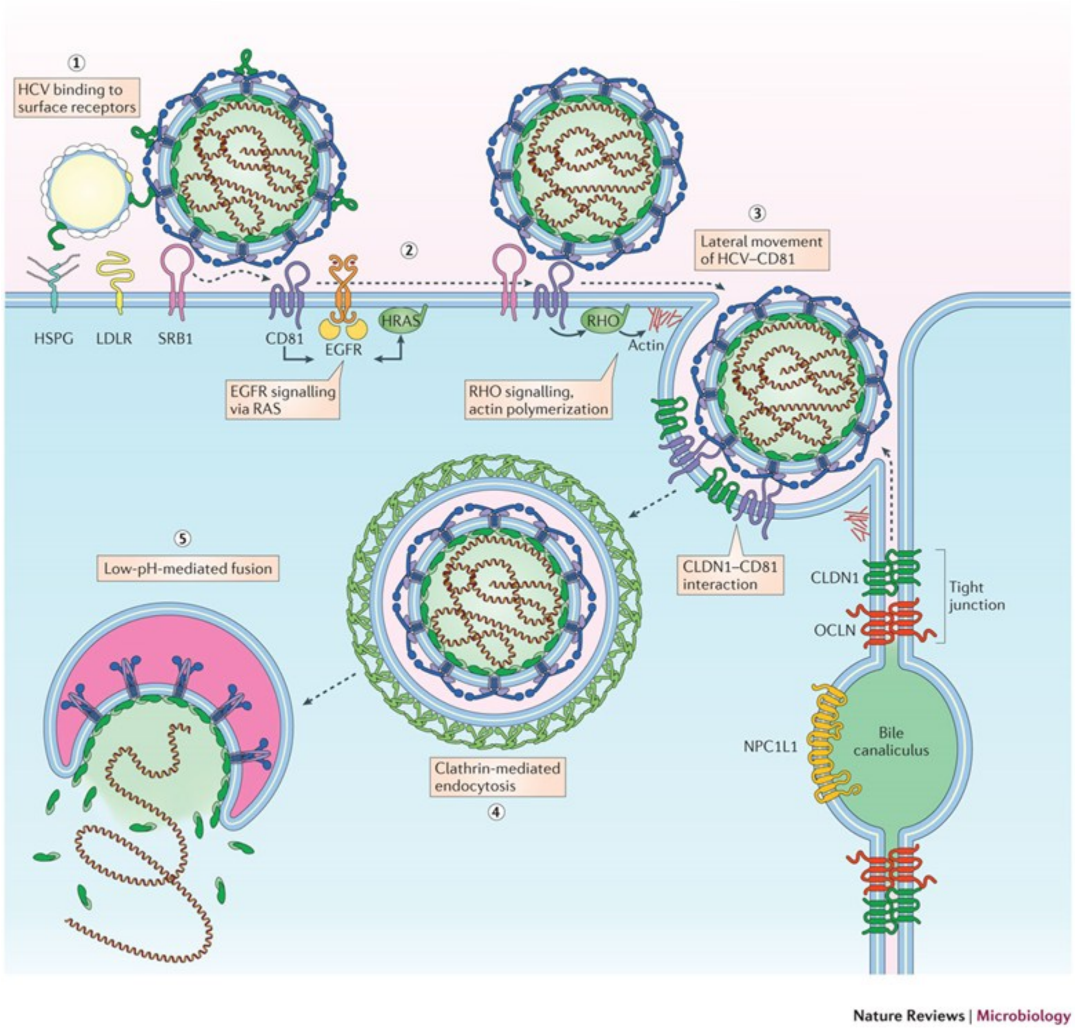
Example: SARS-CoV-2



Stay in line…

Virus entry is a highly controlled process:

- Timely order of (co-)receptor interaction is required
- Lateral movement after binding to the defined microdomains



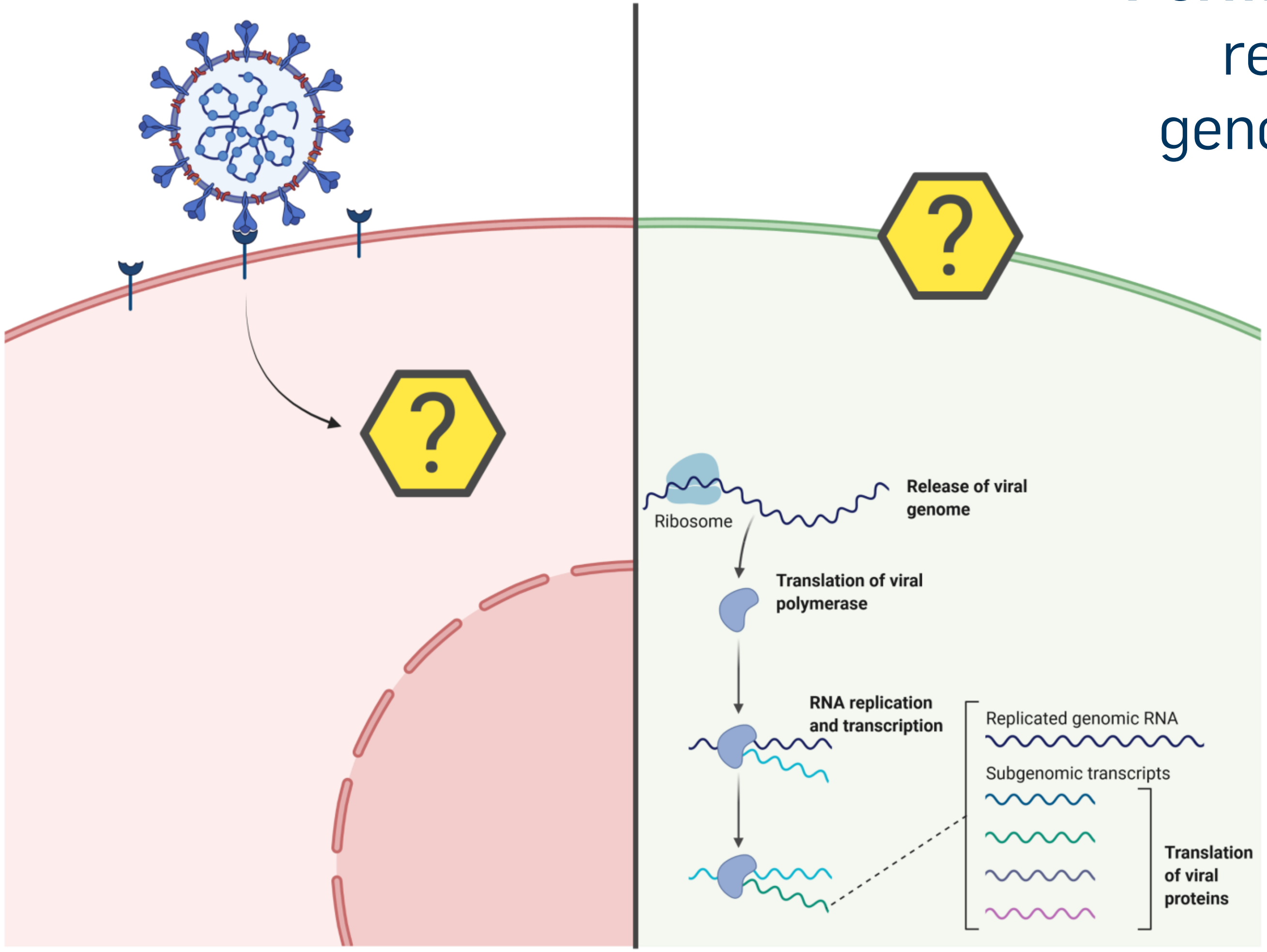
Example: Hepatitis C Virus

Receptor may determine the host range and tissue tropism

Permissive cells support replication of viral genomes (intracellular components)

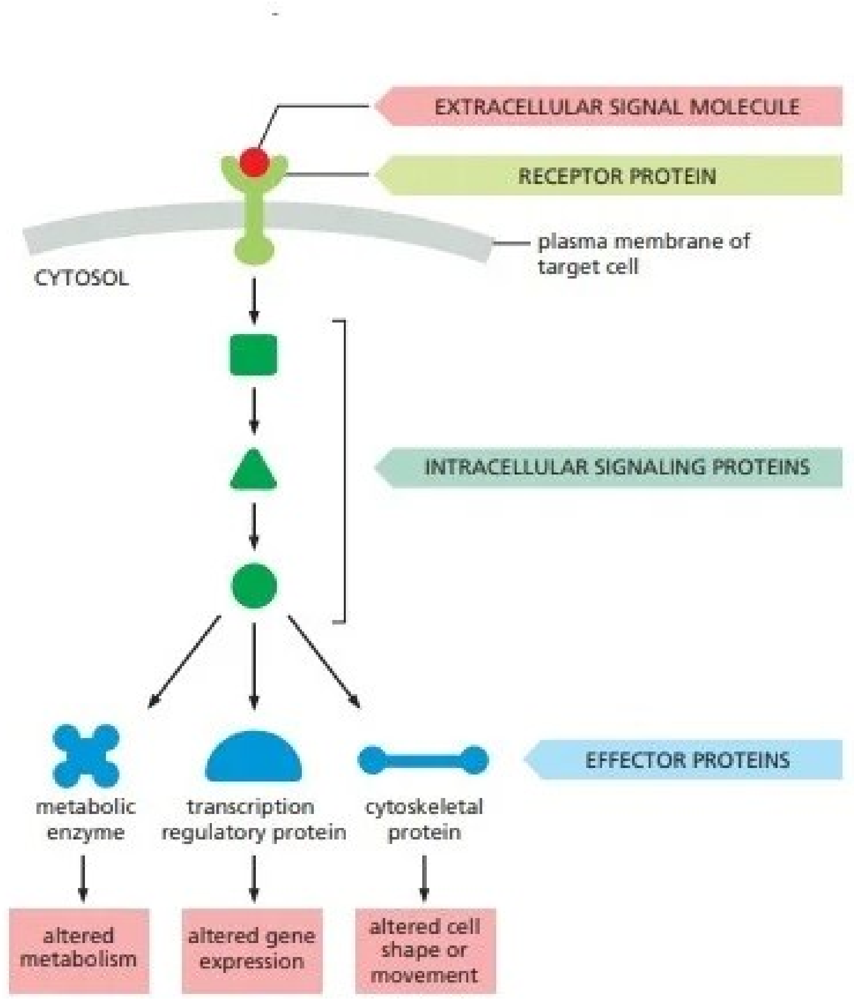
Susceptibility =

capability of a cell to bind and mediate entry of a virus via receptors

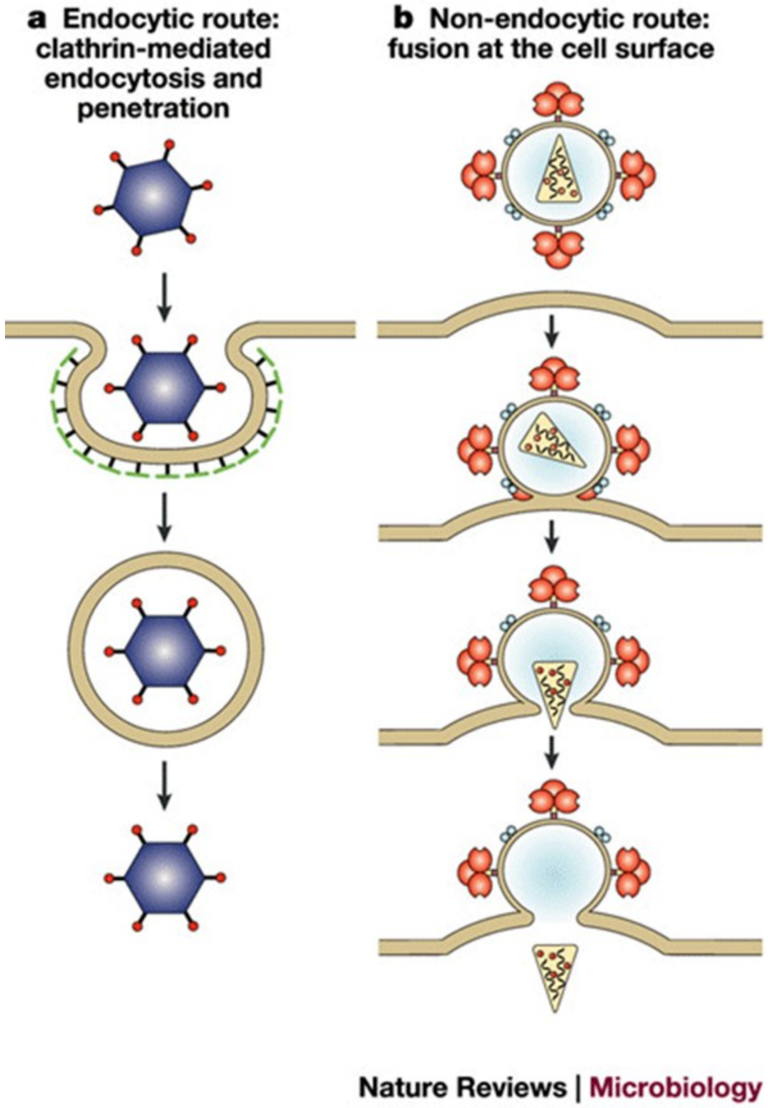


What happens after receptor binding?

- **Binding of a „ligand" to a receptor results in signaling to the cell**
  - adaptor proteins are often kinases which trigger cascades of downstream responses
- **Virus binding „naturally" leads to favourable intracellular conditions**
  - Access to co-receptors, endocytic responses, membrane re-arrangement…

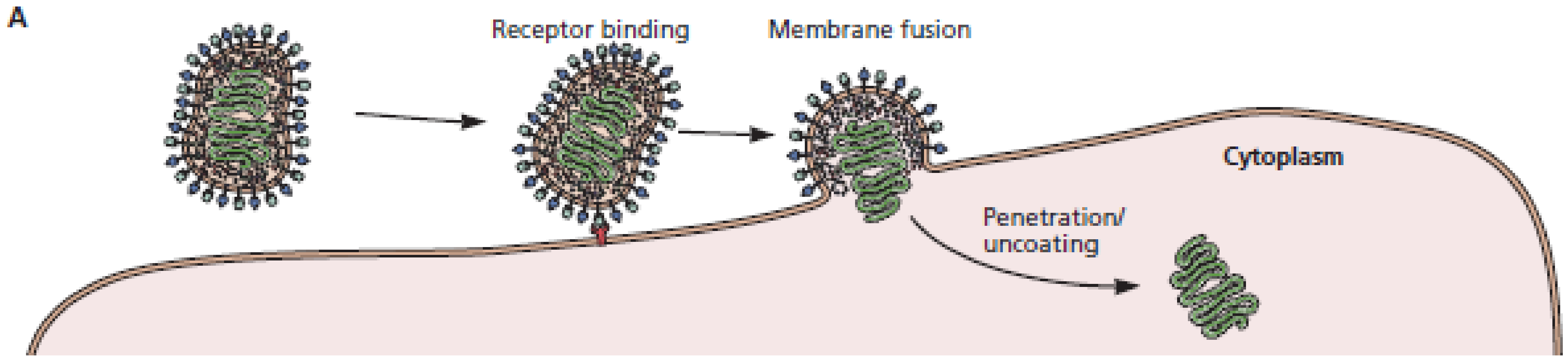


Endocytosis versus Fusion



Uncoating at the plasma membrane via fusion

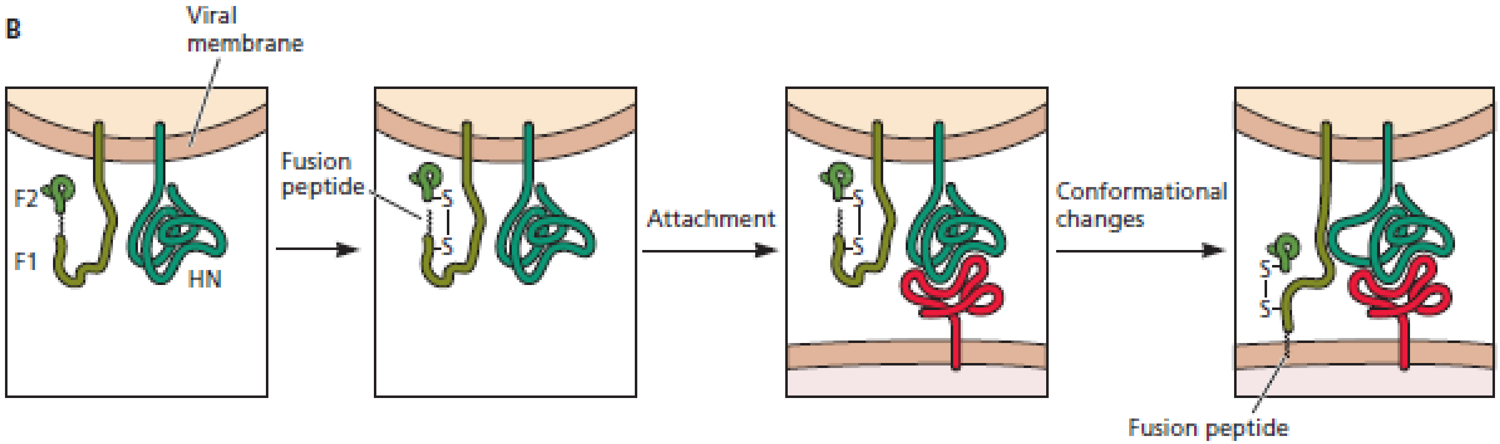
- **The particles of many enveloped viruses fuse directly with the plasma membrane**
  - neutral pH
- **Binding to a cell surface recptor via a viral integral membrane protein**
  - viral and cell membrane are closely juxtaposed
- **Fusion is induced by a second viral glycoprotein = fusion protein (F protein)**



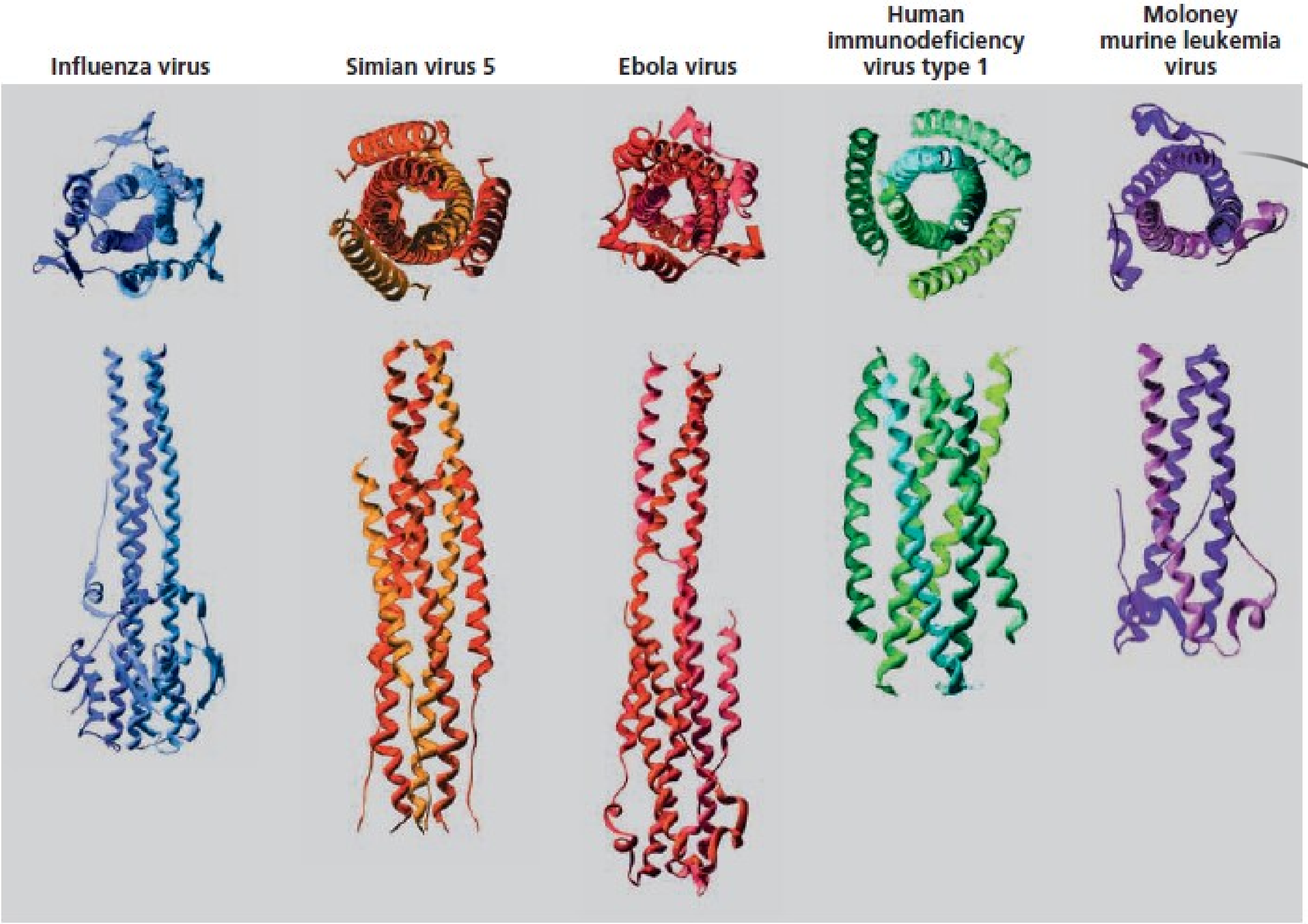
Fusion protein

Examples: HIV
Paramyxoviridae
(Measles, Hendra, Mumps virus)
Herpesviridae (CMV…)
Poxviridae

- **F proteins are type 1 integral membrane glycoproteins**
  - N terminus lies outside the viral membrane
  - Homotrimer
- **Precursor called F0**
  - Cleavage by host cell protease (F1 and F2, connected by a disulfide bond) is indispensable
  - F1 = fusion peptide, inserts into the target membrane
- **Highly controlled by conformational changes of the F protein**



Similarities among viral fusion proteins



Central three-stranded coiled coil supported by C-terminal structures

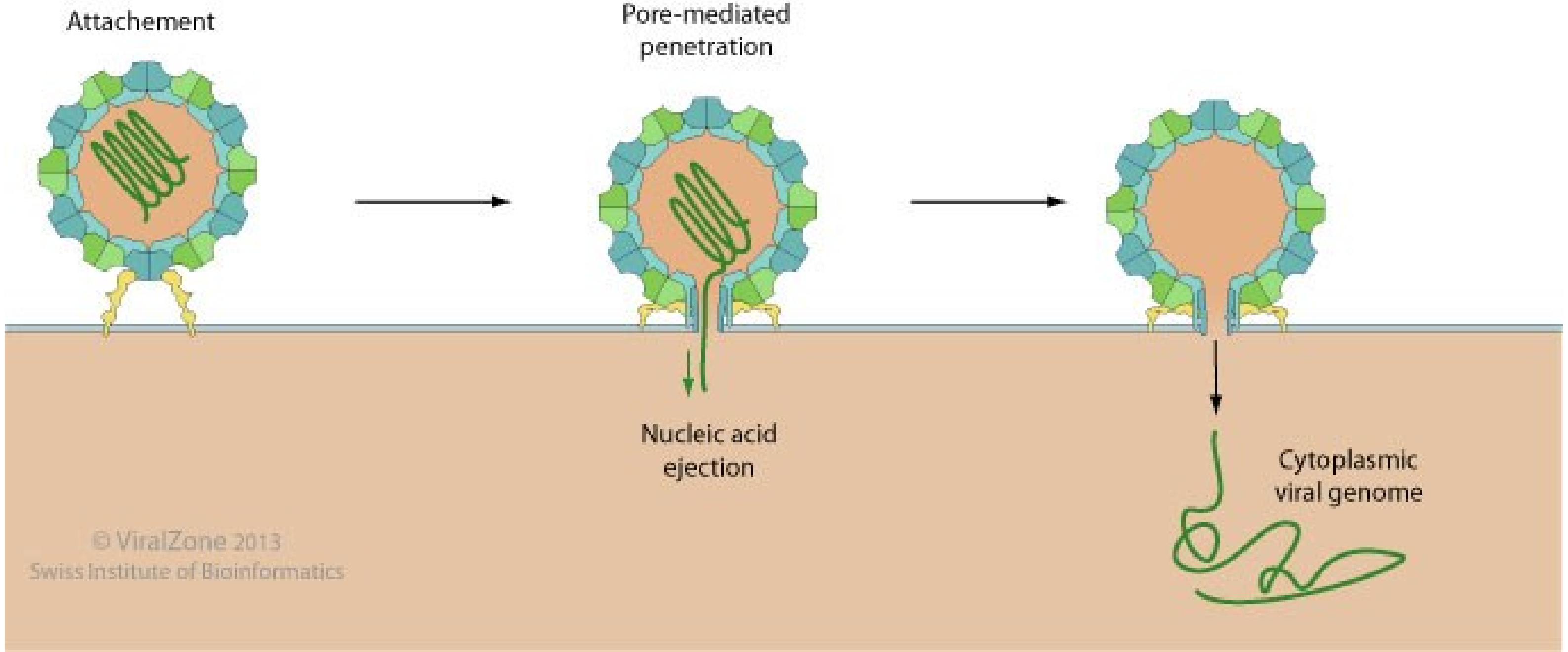
And what's about nonenveloped viruses?

Membrane puncture:

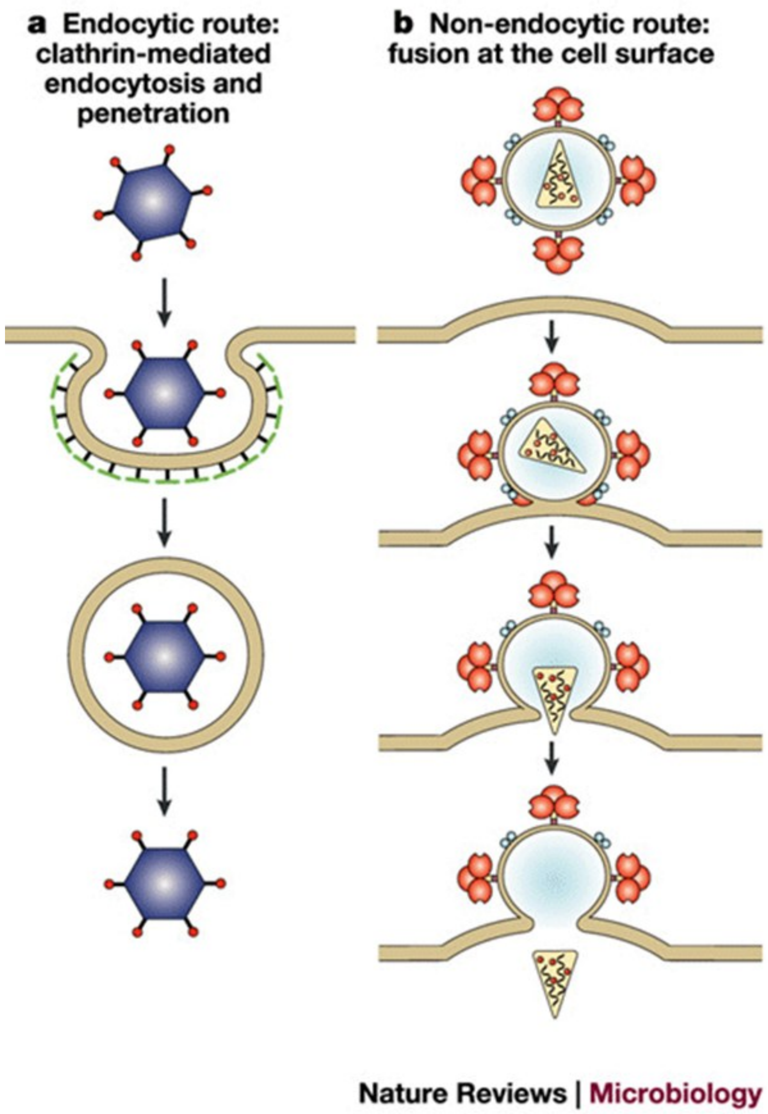
The virus particle generates a pore in the membrane

- Genome is selectively released to the cytosol
- The viral capsid does not enter the cell

Example: Picornavirus



Endocytosis versus Fusion



Endocytic pathways for virus entry

a. Clathrin-mediated

- Most commonly used mechanism
  (VSV, SFV, DENV, Rhinovirus, IAV, Adenovirus 2/5

b. Caveolin-mediated

- Caveolin and lipid rafts are involved
- Mainly used by the polyomavirus SV40

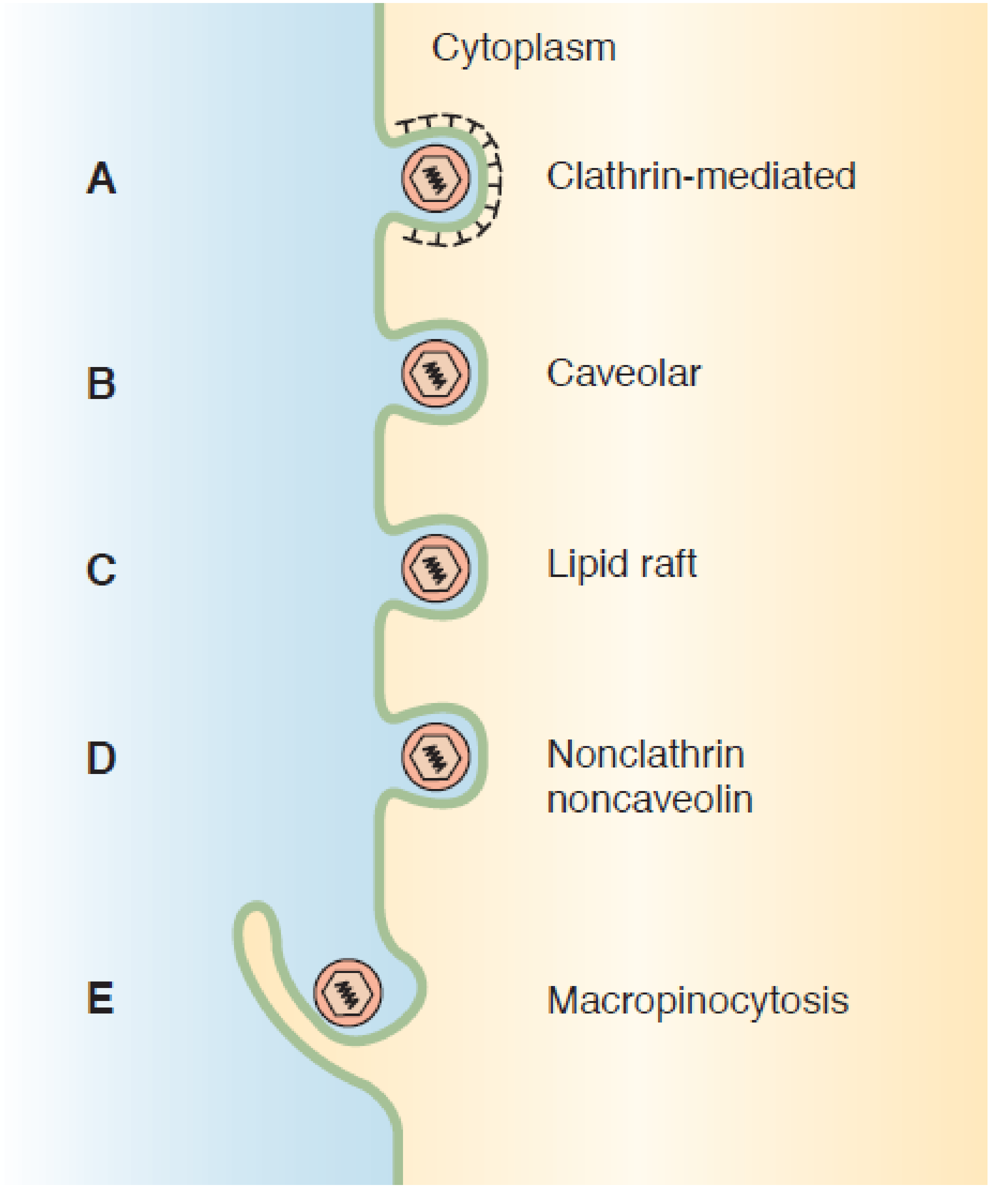
c. Lipid raft-dependent

- Many similarities to the caveolar mechanism
- Used by many polyomaviruses

d. Non-clathrin, non-caveolin

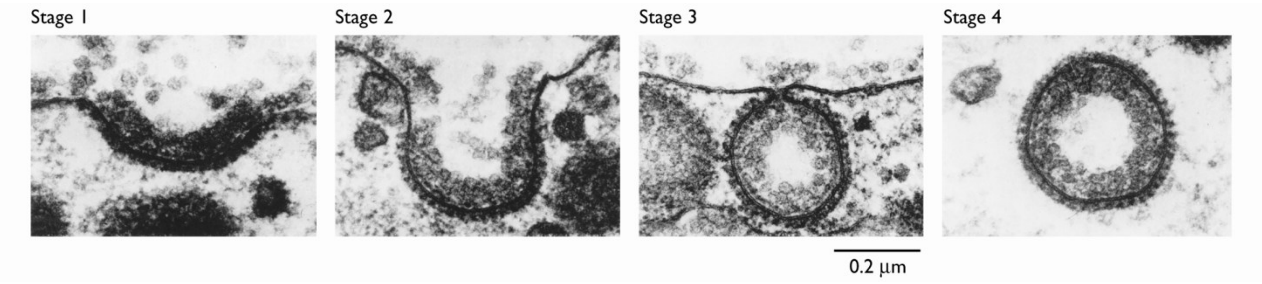
e. Macropinocytosis

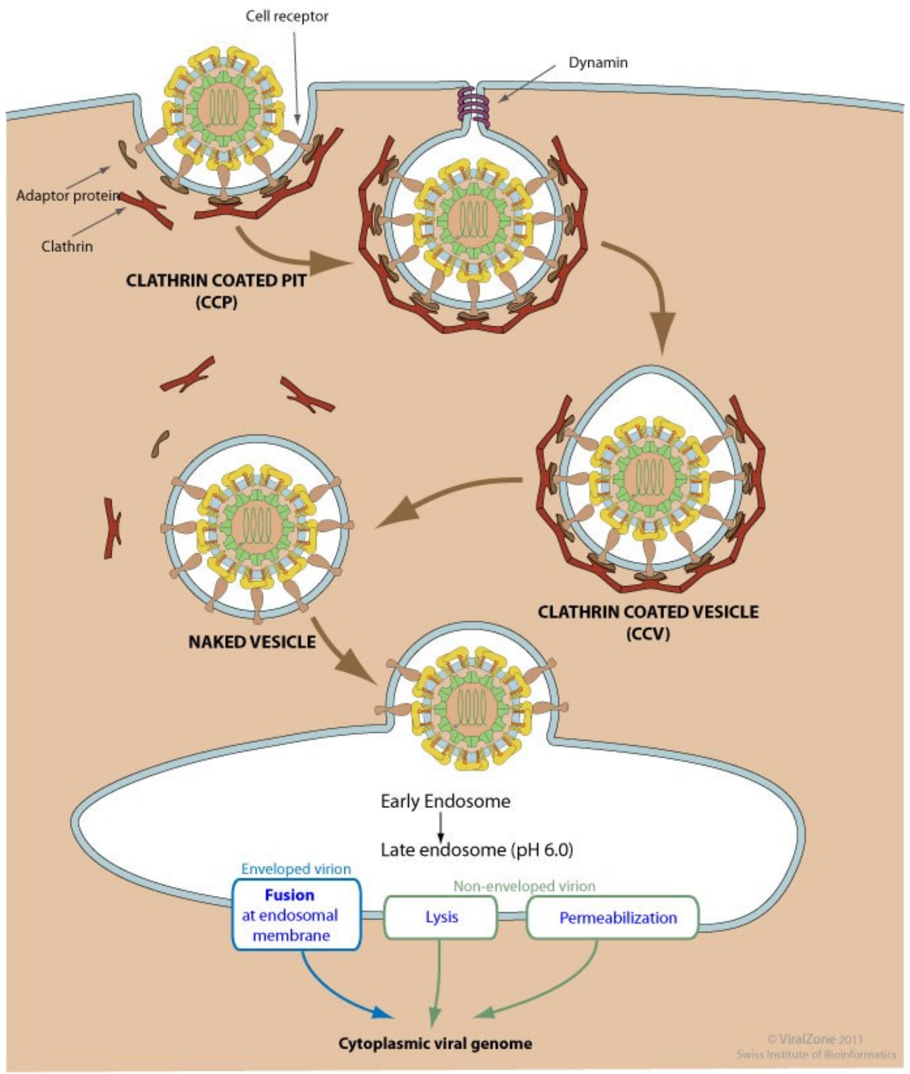
- Transient, ligand induced, actin-dependent mechanism



Clathrin-mediated endocytosis (CME)

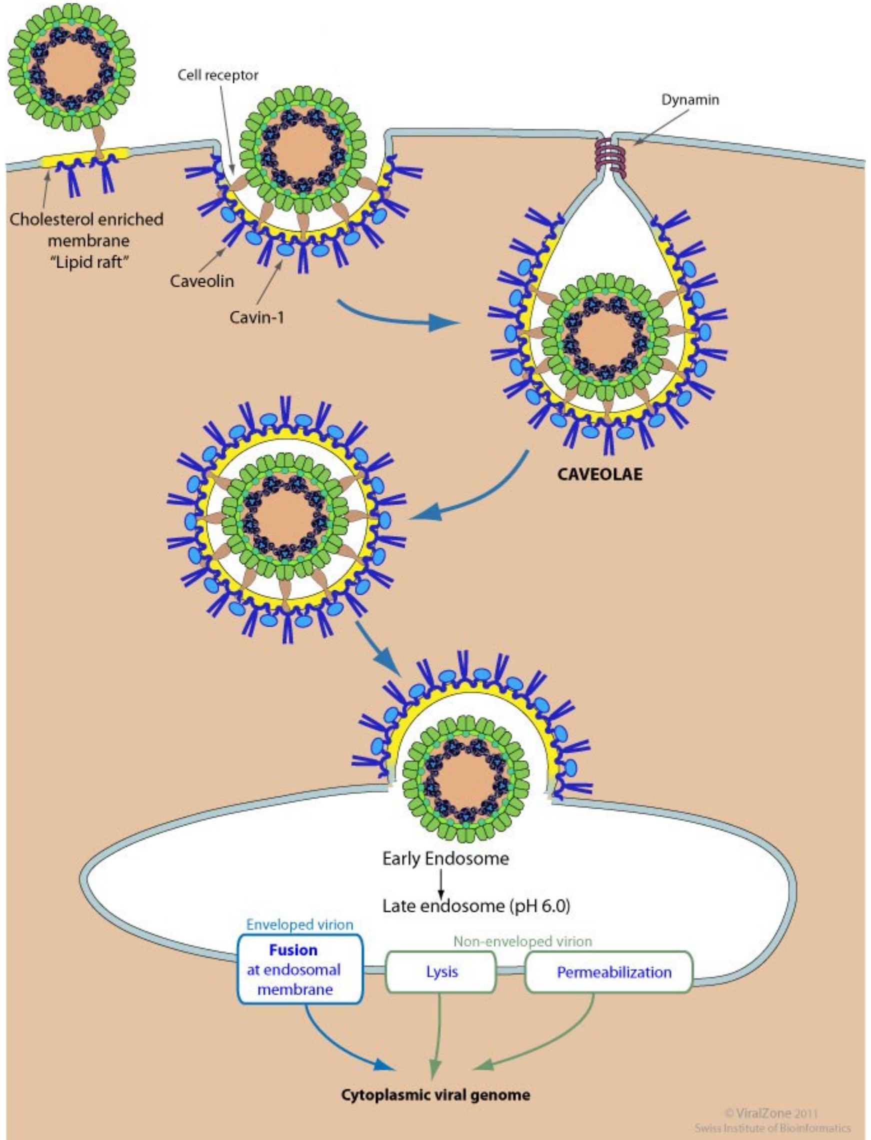
- **Cells use CME to internalize a broad spectrum of receptor-bound ligands, fluid, membrane proteins and lipids for recycling and degradation**
  - Viruses = opportunistic ligands
- **Rapid kinetics (few minutes)**
- **high capacity (3,000 virus particles)**
- **120 nm diameter**
  - enables uptake of large particles (VSV)
- **Most CCV form *de novo* under the virus particle**
  - Only a few viruses enter via pre-existing vesicles





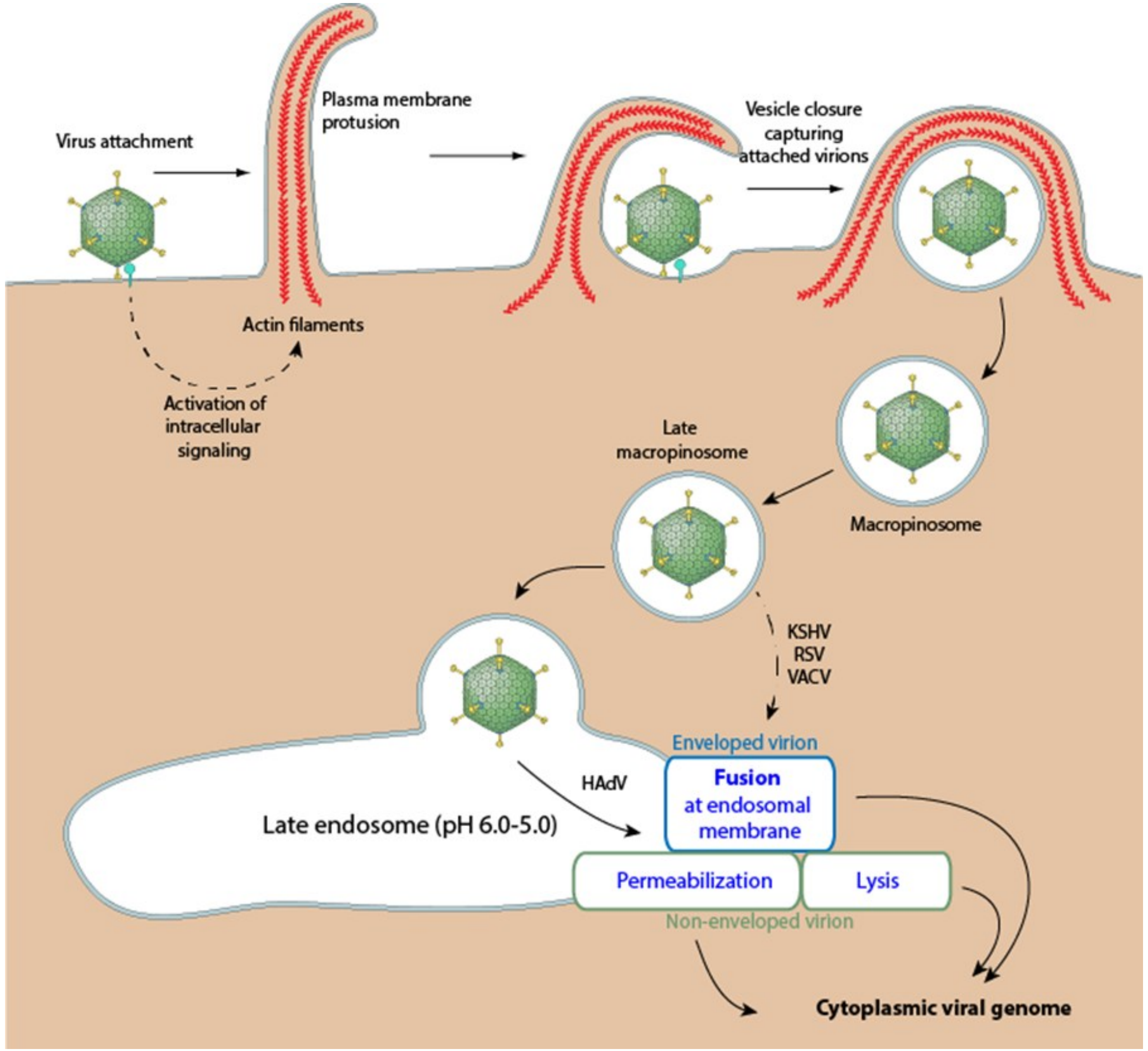
Caveolin-mediated endocytosis

- **First observed for Polyomaviruses**
- **Caveolins**
  - integral membrane proteins that bind directly to membrane cholesterol
- **Flask-like shape of the caveolae (70 nm)**
  - Caveolins, cavins, ernriched in cholesterol and sphingolipis



Macropinocytosis

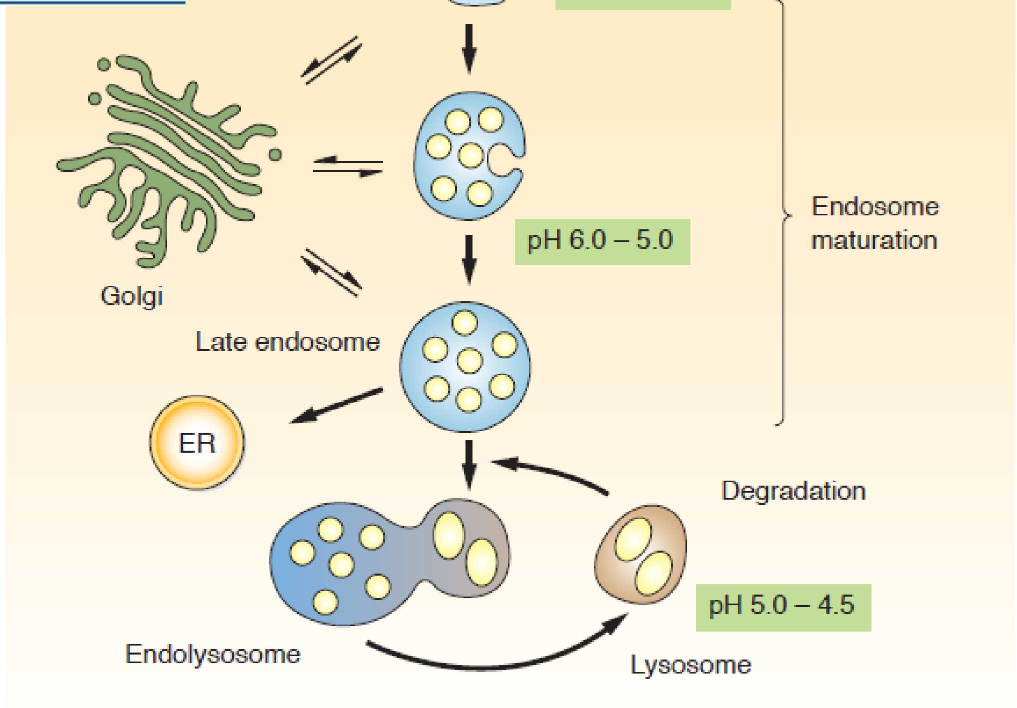
- **Commonly used by larger viruses**
  - Vaccinia virus, HSV-1, Adenovirus 3, Kaposi's sarcoma virus, RSV
  - Mostly non-enveloped viruses
- **Ligand triggered, transient, actin dependent**
- **Physiological cargo = extracellular fluid**
- **Activation of signaling cascade leads to changes in cortical actin and ruffling of plasma membrane**
- **Differs from phagocytosis**
  - Signaling cascade
  - Fails to inactivate innate immune response + inflammation



The endosomal pathway

- **Two interconnected cycles of membrane tracking**
  - Endosome
  - Lysosome
- **Endosome:** sorting and recycling of incoming membrane components, ligands and fluid back to the cell surface
- **Lysosome:** degradation of receptor-ligand complexes, degradation and processing of nutrients, digestions autophagic substrates
- **Virus get a free ride through the cortical cytoskeleton and other barriers that hinder movement of virus-sized particles in the cytoplasm**
  - Specific enviroment (low pH, proteases) helps with penetration/uncoating
  - Immunrecognitions is delayed = no traces

Low pH-dependent	pH-independent
Adeno	Corona (majority)
Alpha	Retroviruses (majority)
Borna	Herpes (majority)
Bunya	Paramyxo
Corona (some)	Hepadna
Filo	Pox (some)
Flavi	Rota
Orthomyxo	Picorna (most)
Parvo	Noro
Papilloma	
Picorna (some)	
Pesti	
Pox (some)	
Rhabdo	
Arena	
Arteri	
Hepaci	

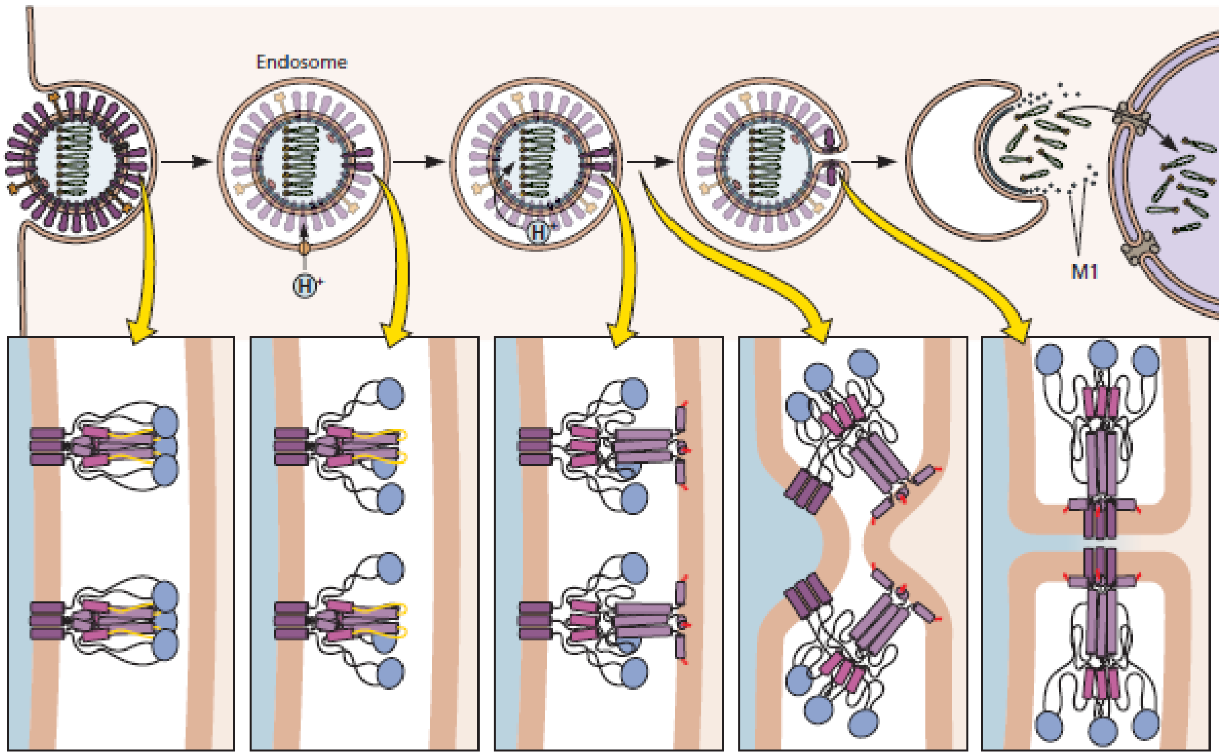


How do viruses escape from the endocytic pathway?

Acid-catalyzed membrane fusion

Example: Influenza virus

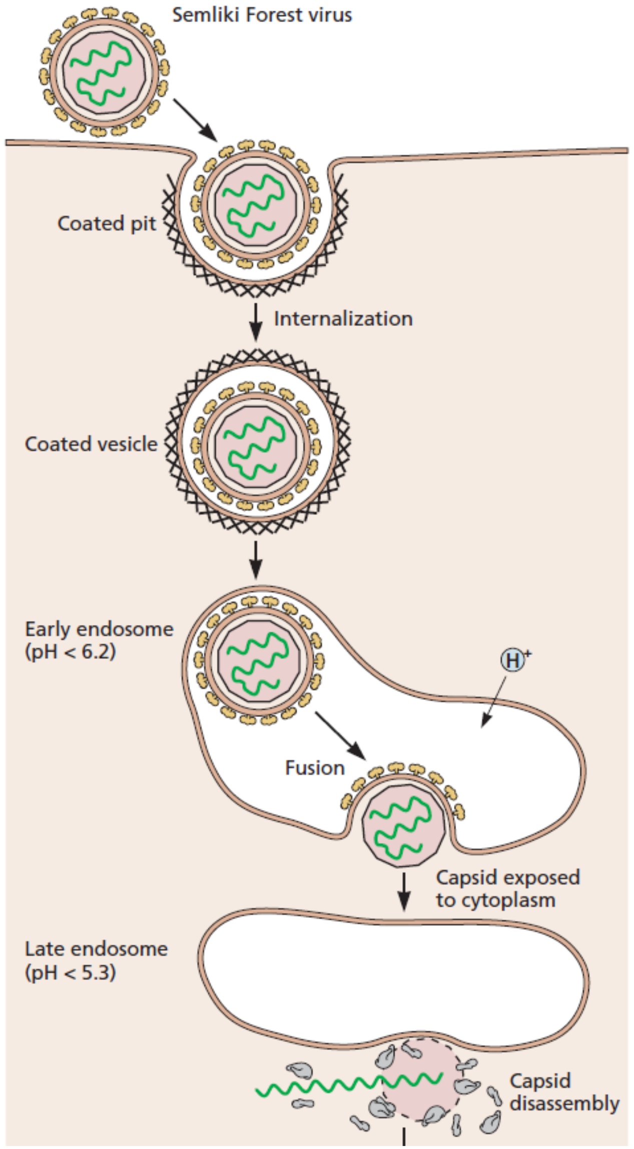
- acidification (pH 5) leads to conformational changes of the HA protein = hairpinning
- Fusion peptide inserts in the cell membrane
- Fusion through bending of the complex
- M2 = ion channel
  - H+ ions are pumped into the particle
- vRNP dissociates from the matrix protein M1
  - Release of the viral RNP into the cytoplasm = penetration



Acid-catalyzed membrane fusion

Example: Semliki Forest Virus

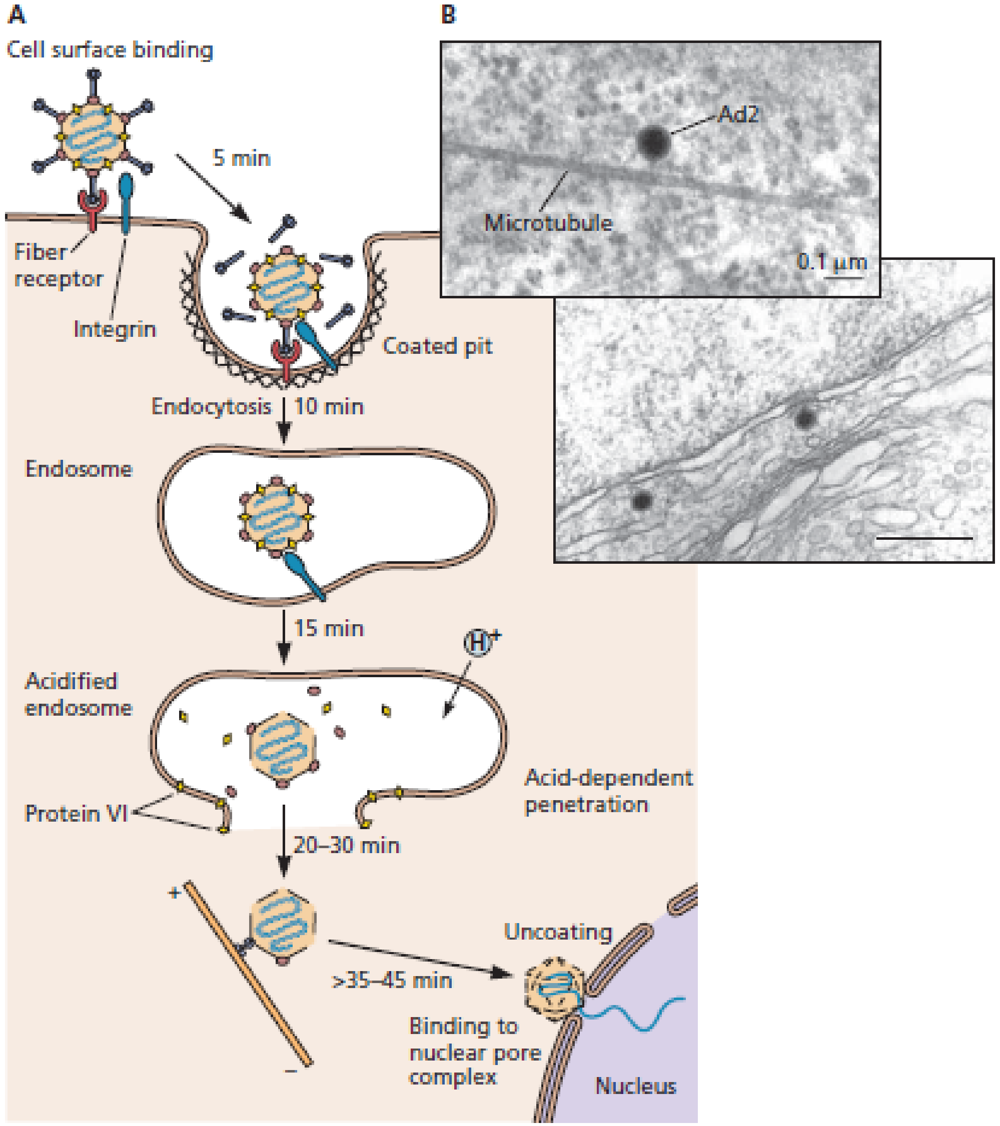
- Entry via clathrin-dependent endocytosis
- Membrane fusion is catalyzed by acidification of endosomes
- Fusion results in release of the nucleocapsid to the cytoplasm
- Nucleocapsid is disassembled in the cytoplasm by pH-independent mechanisms
  - Cellular ribosomes process the capsid prior to translation and replication
  - Still attached to the cytosolic side of the late endosome



Acid-catalyzed **penetration**

Example: Adenovirus

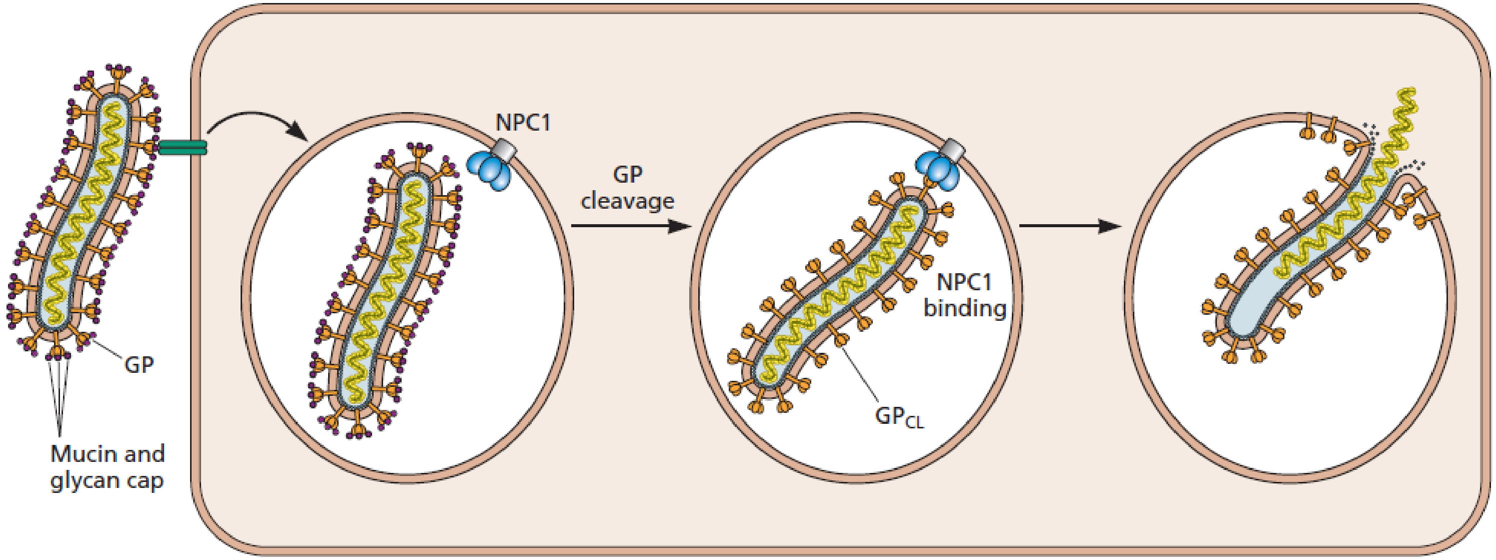
- Receptor: fiber protein
- Internalization by clathrin-mediated endocytosis
- Low pH in the endosome causes destabilization of the capsid
- Released V1 protein disrupts the endosomal membrane
  - Release of the subviral particle
  - Transported to the nucleus for uncoating



Protease-catalyzed membrane fusion

Example: Ebolavirus

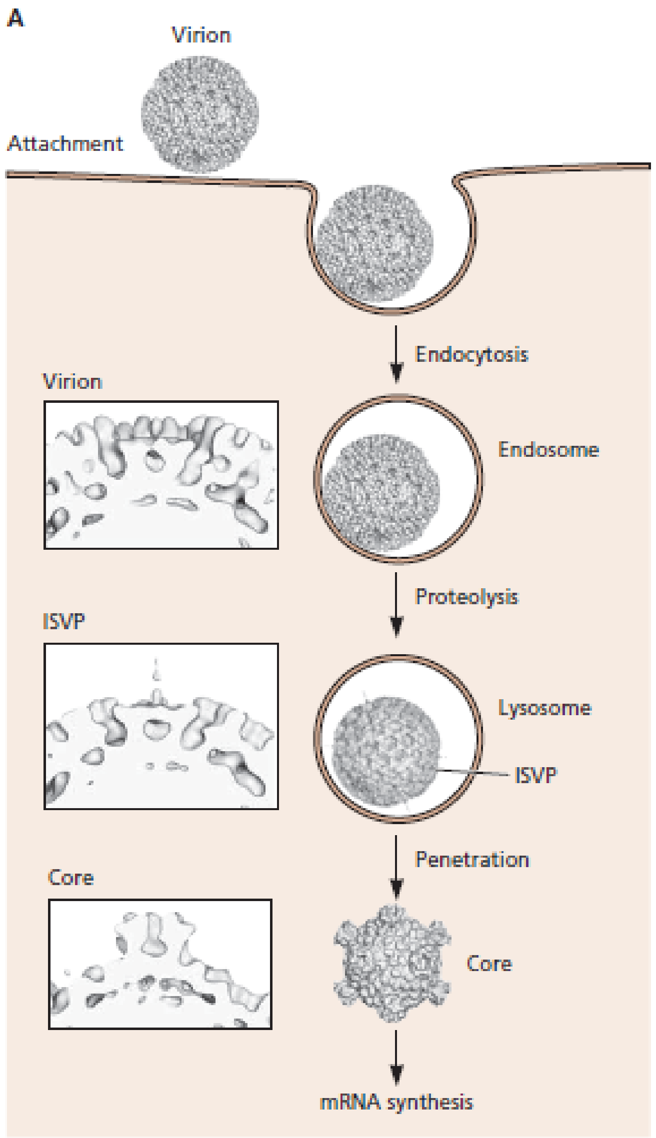
- Receptor: unknown
- Entry via endocytosis
- Mucin and glycan caps on the viral glycoprotein (GP) is removed by cysteine proteases
- Exposing binding sites for NPC1
- GP-NPC1 interaction results in membrane fusion and release of the nucleocapsid



Special effects: uncoating in the lysosome

Example: Reovirus

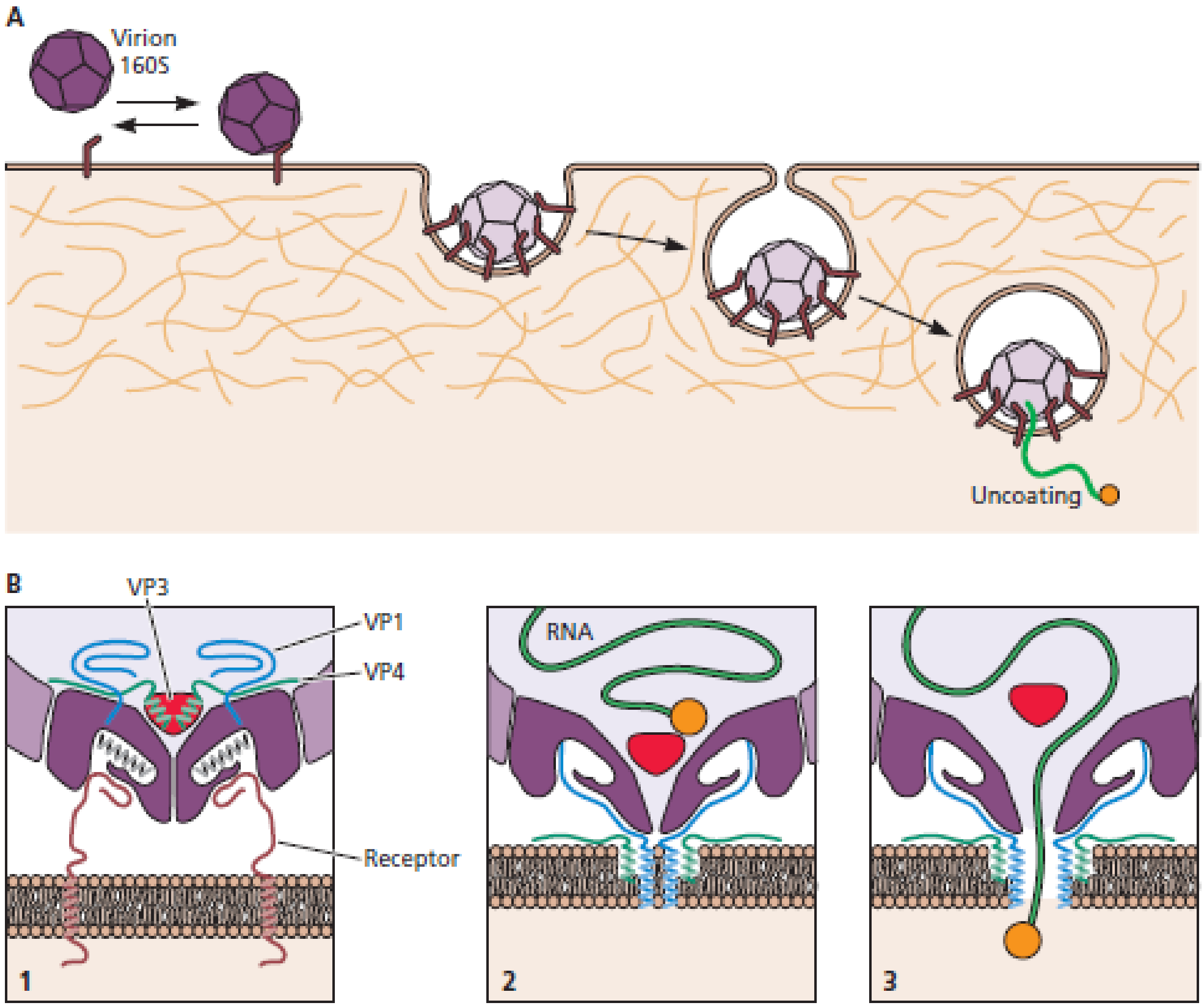
- Receptor: unknown
- Entry via endocytosis
- Proteolysis in the late endosome results in an infectious subviral particle
- Further cleavage and release of capsid proteins enable the virus to penetrate the lysosomal membrane
- Fun fact: core particles carry out viral mRNA synthesis



Special effects: forming a pore in the endosomal membrane

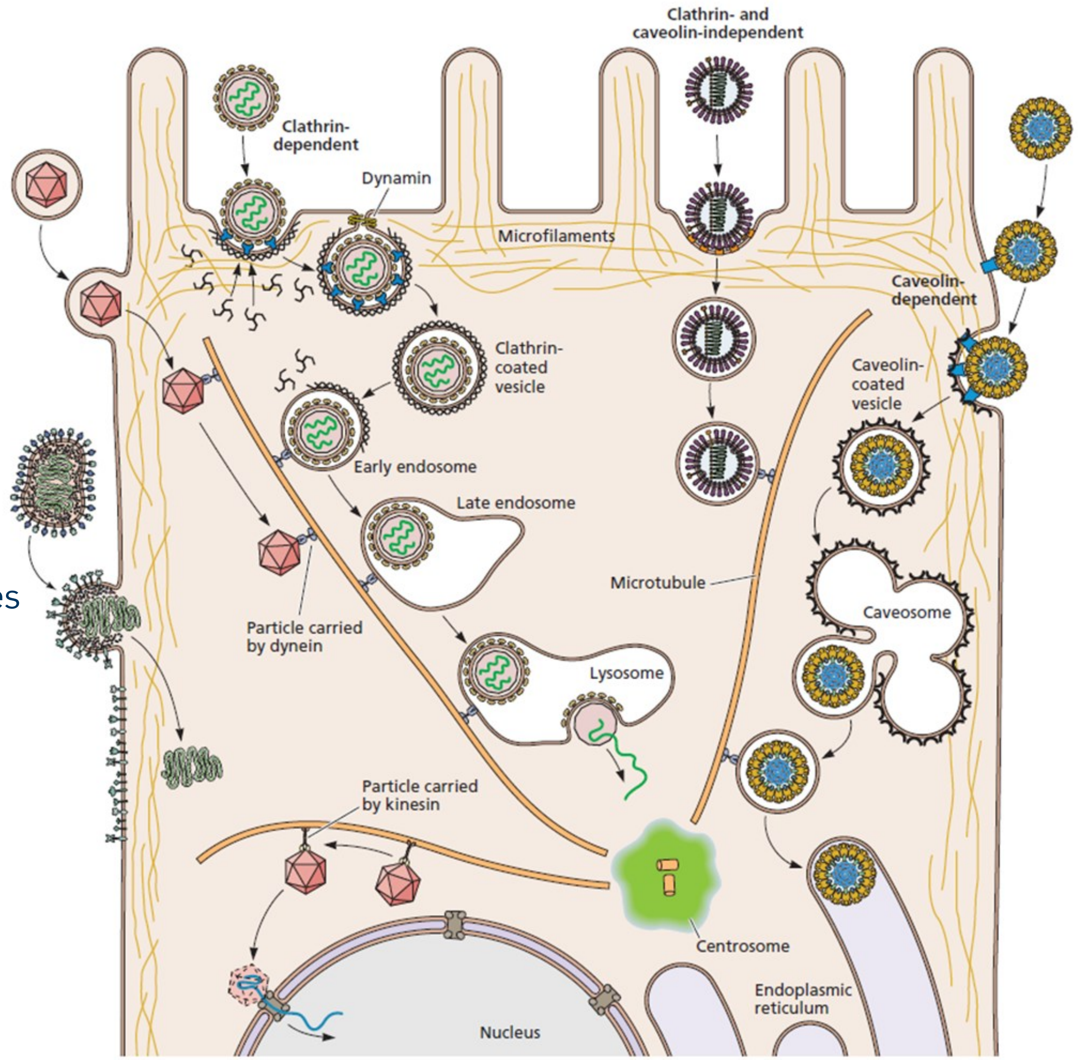
Example: Poliovirus

- Receptor: CD155
- Native virion binds to the receptor
  - Undergoes receptor-mediated conformational transition to an altered particle
- Viral RNA leaves from the early endosome by pore formation
  - Portions of two capsid proteins VP1 and VP4 insert into the endosomal membrane
  - formation by a hdyrophobic tunnel



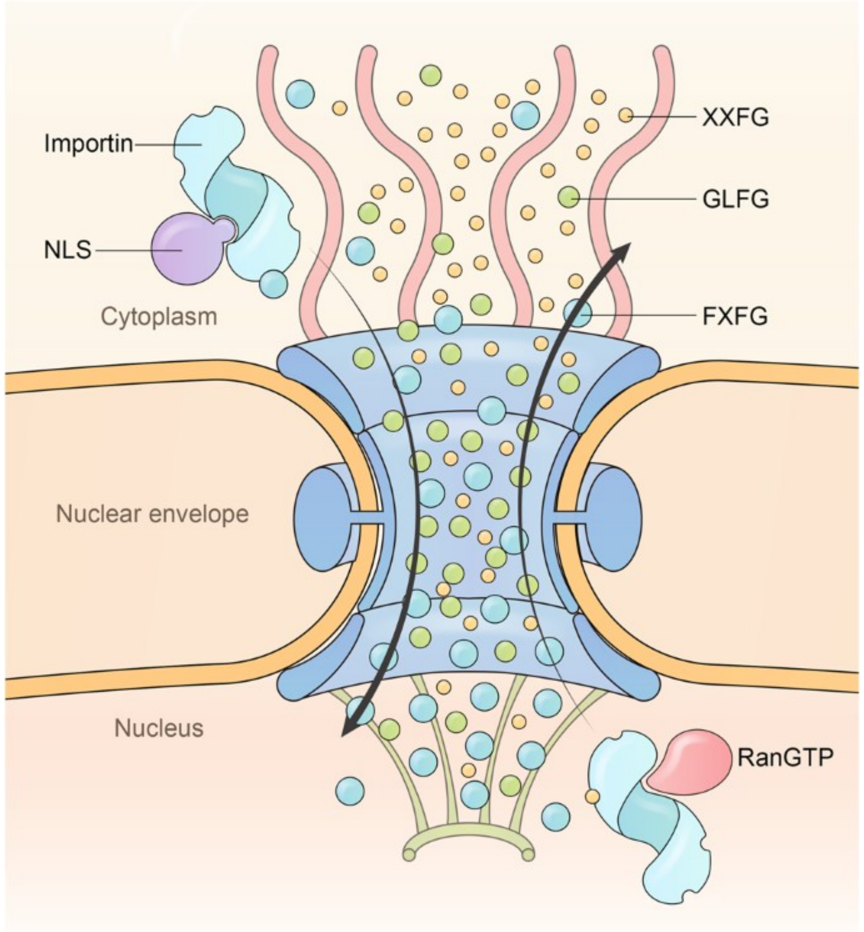
Movement of viral particles within cells

- **Movement of molecules > 500 kDA does not occur by passive diffusion**
- **Cytoplasm is full of organelles, proteins and the cytoskeleton**
- **Viruses either get a free ride via**
    1. Endosomes
        - Movement of endocytic vesicles occurs on microfilaments or microtubules
        - Penetration/uncoating at the site of viral replication/translation
    2. Or directly bind to the transport machinery
- **RNA viruses predominantly replicate in the cytoplasm**
- **DNA viruses predominantly replicate in the nucleus**



Import of viral genomes into the nucleus

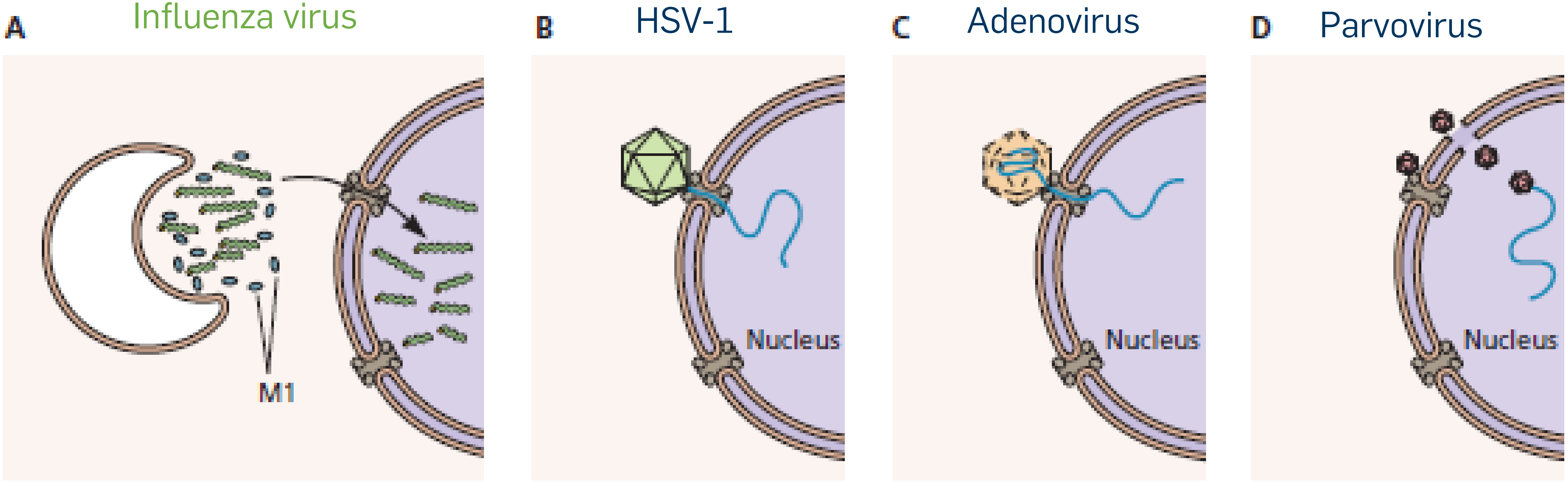
- **Most DNA viruses and some RNA viruses (retroviruses and influenza viruses) replicate in the nucleus**
- **Genome must be imported from the cytoplasm**
  1. Cellular pathway using the nuclear pore transport
     - Nuclear localization signal (NLS) is necessary
  2. Nuclear envelope breaks down during cell division (mitosis)
     - Viral DNA + cellular chromatin is incorporated when nuclear envelope is reformed



Different strategies to enter the nucleus

RNA virus

DNA viruses



Each segment is small enough to be transported through the NPC.

Capsid docks to NPC and is minimally disassembled to allow transit of viral DNA into nucleus.

Subviral particle is dismantled at NPC, allowing transport of viral DNA into nucleus.

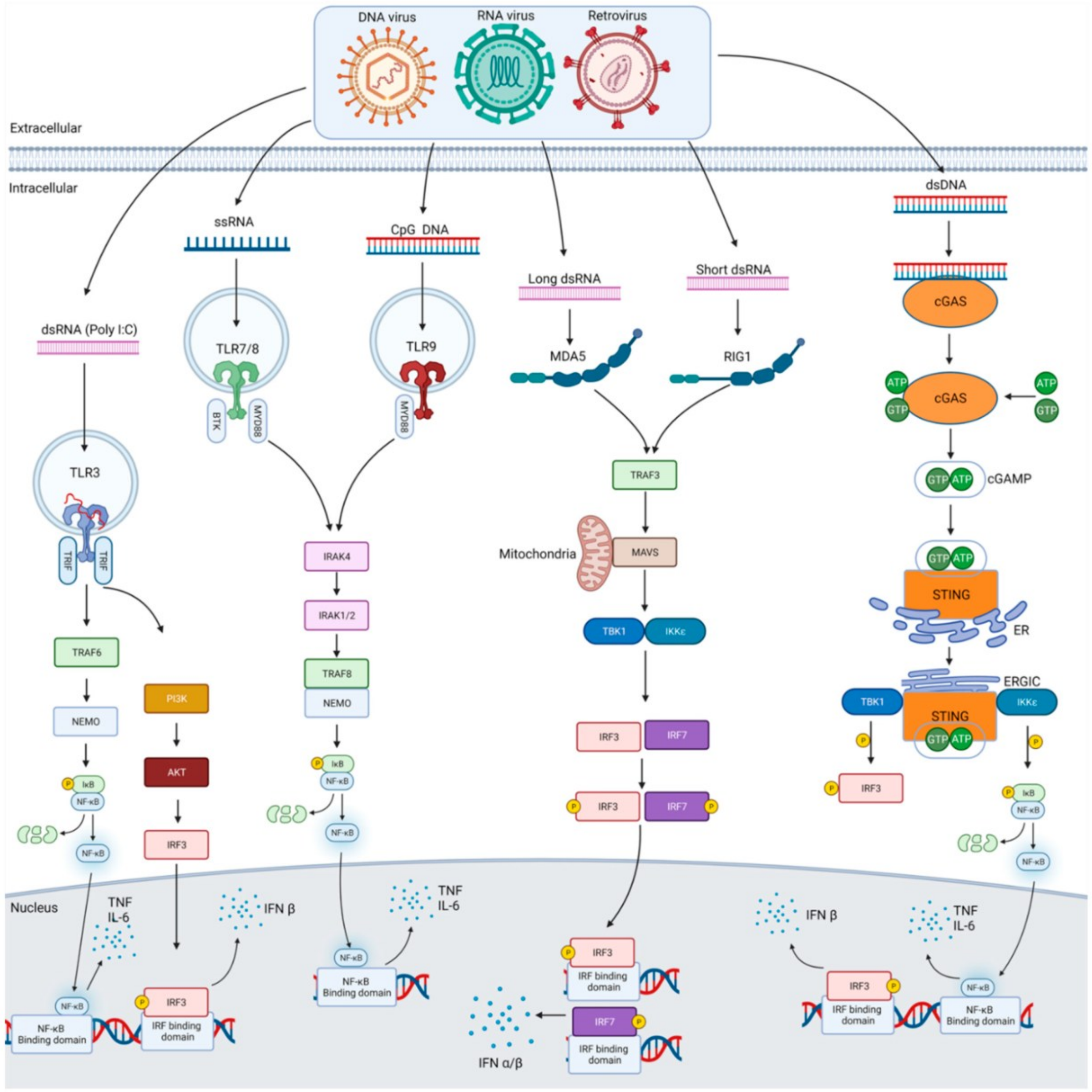
Virus particles bind to NPC, which causes disruption of the nuclear envelope followed by nuclear entry.

Nuclear pore complex (NPC)

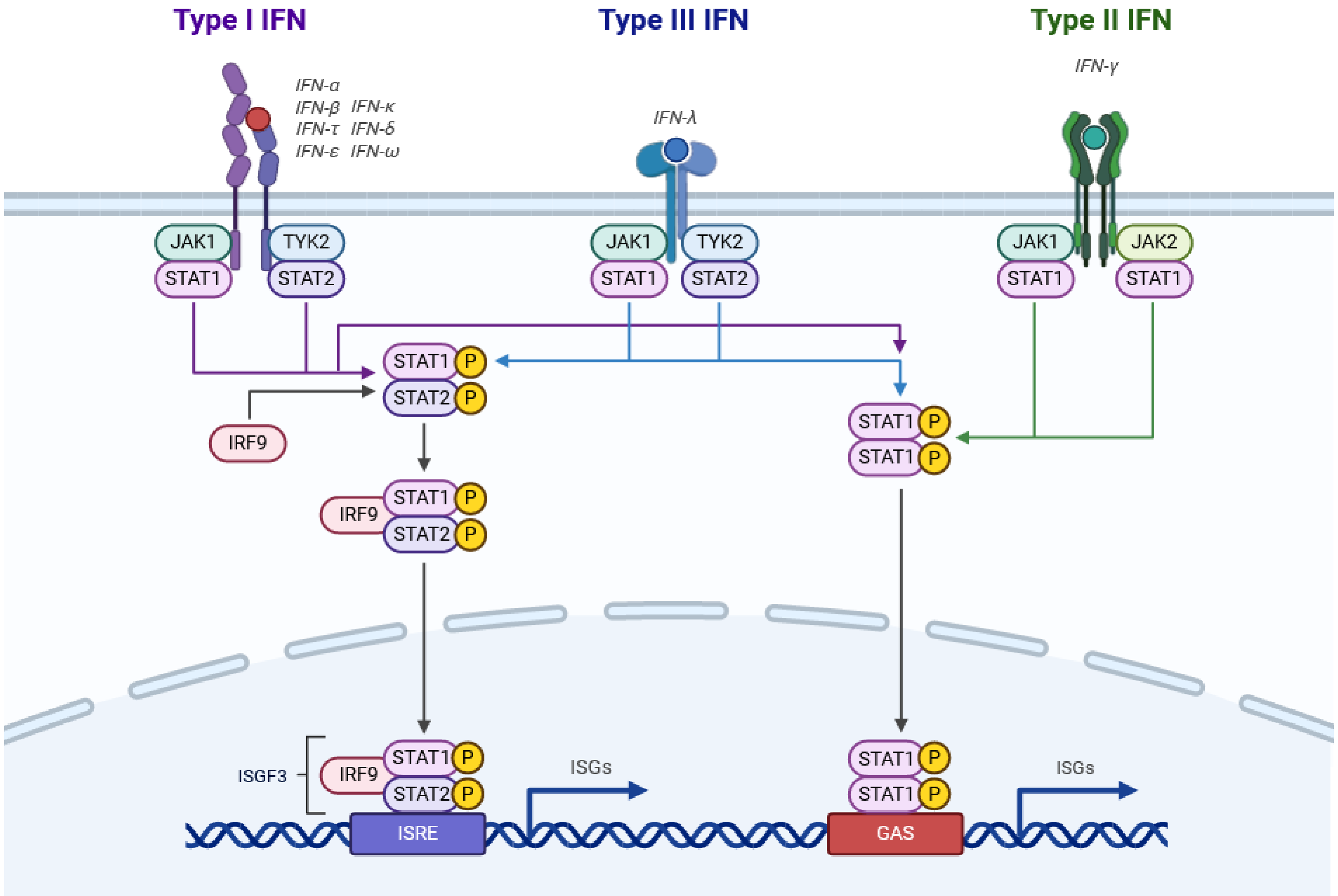
Molecular & Medical Virology

Detection of viral infection by innate immunity

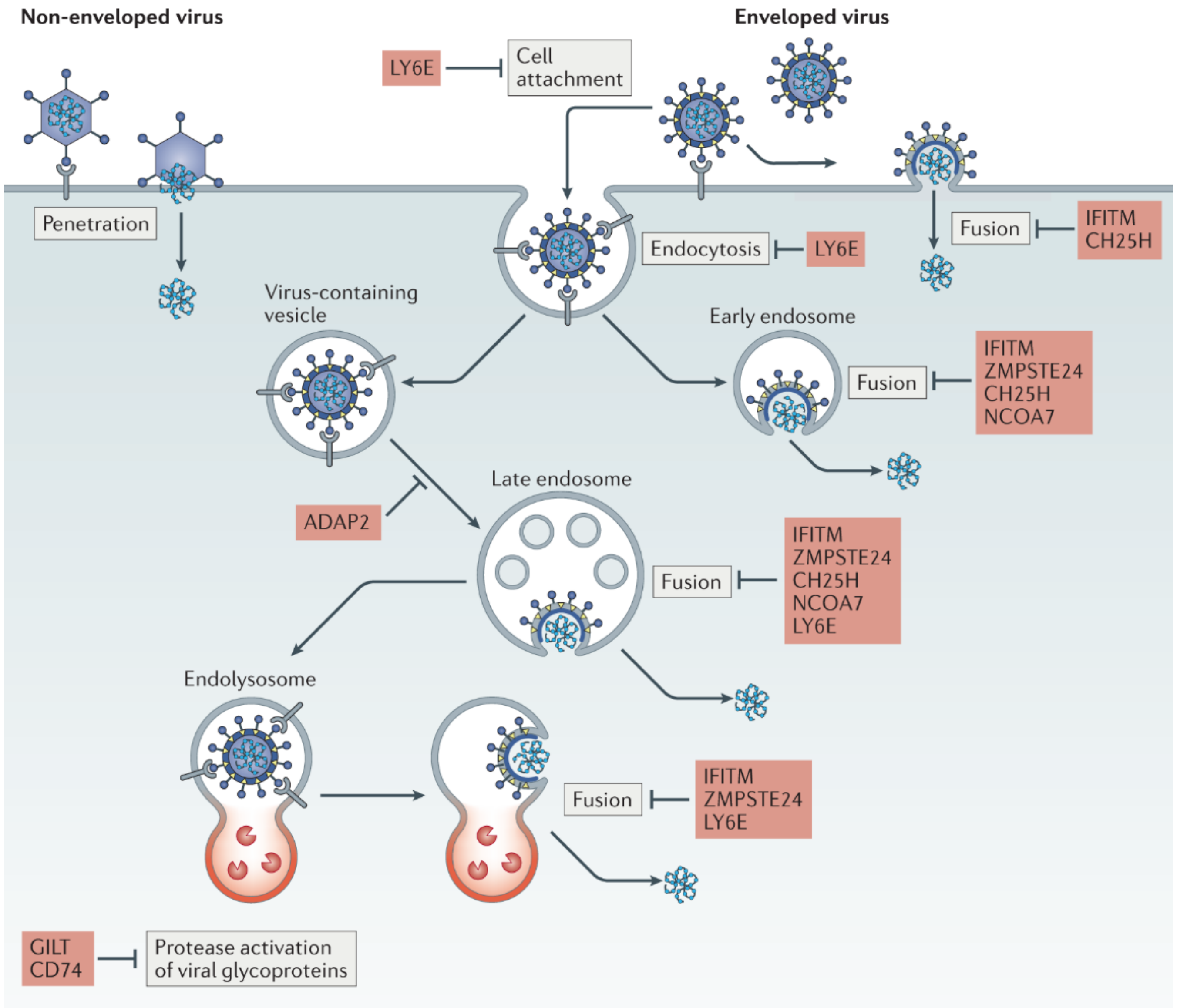
- **Virus infection is sensed by recognition of viral nucleic acids**
  - Pathogen associated molecular pattern (PAMP)
  - Detected by pattern recognition receptors (PRRs)
- **Activation of complex signalling cascades results in secretion of type I and type III interferons (IFNs), chemokines and pro-inflammatory cytokines to activate inflammation**



Interferon stimulated genes



Interferon stimulated genes: What do they all do?



Why do we need to know the receptor?

?

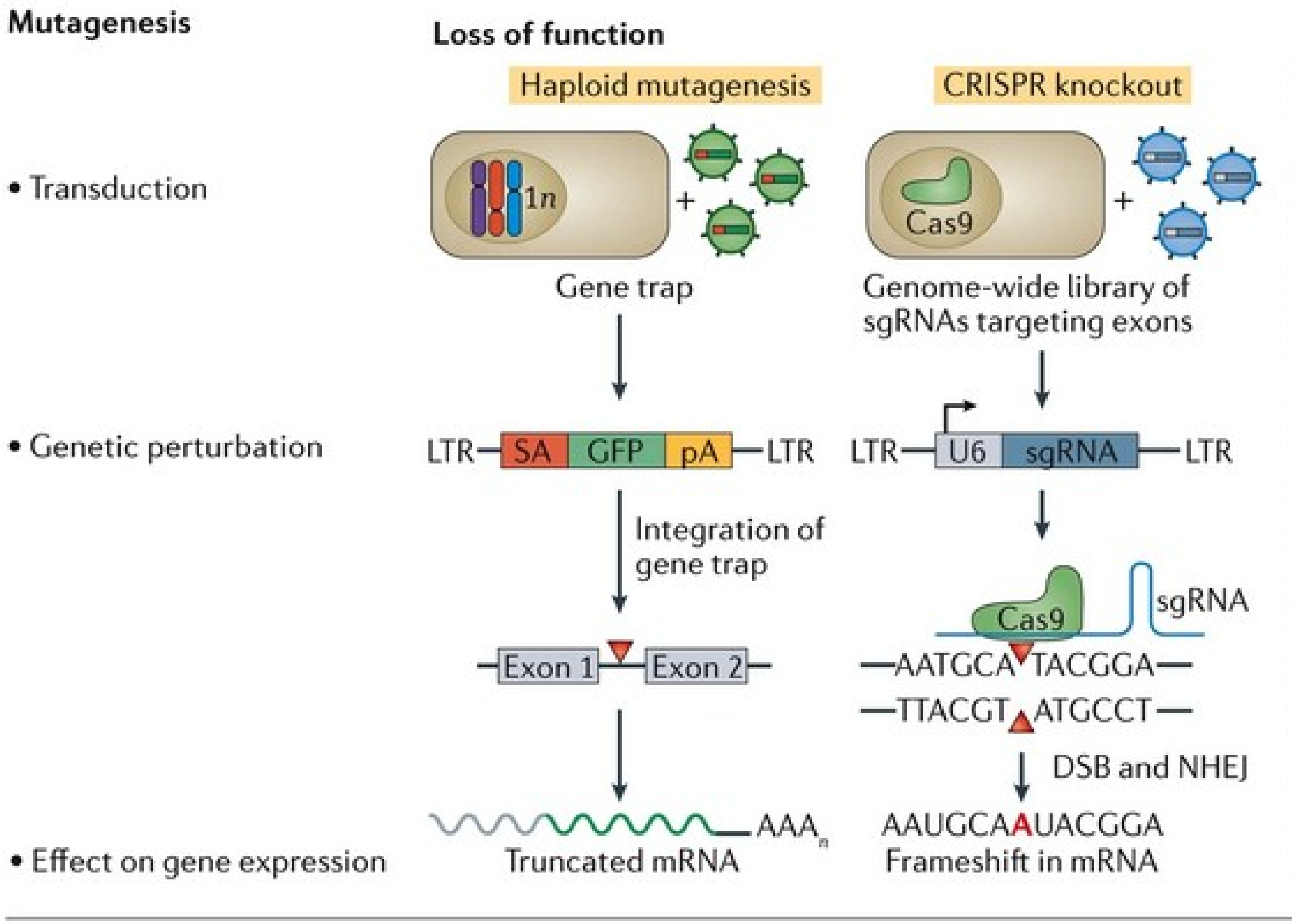
Therapeutic Approach

Infection prevention

Tropism – Symptoms – Zoonotic potential

Risk assessment

How to identify receptors?

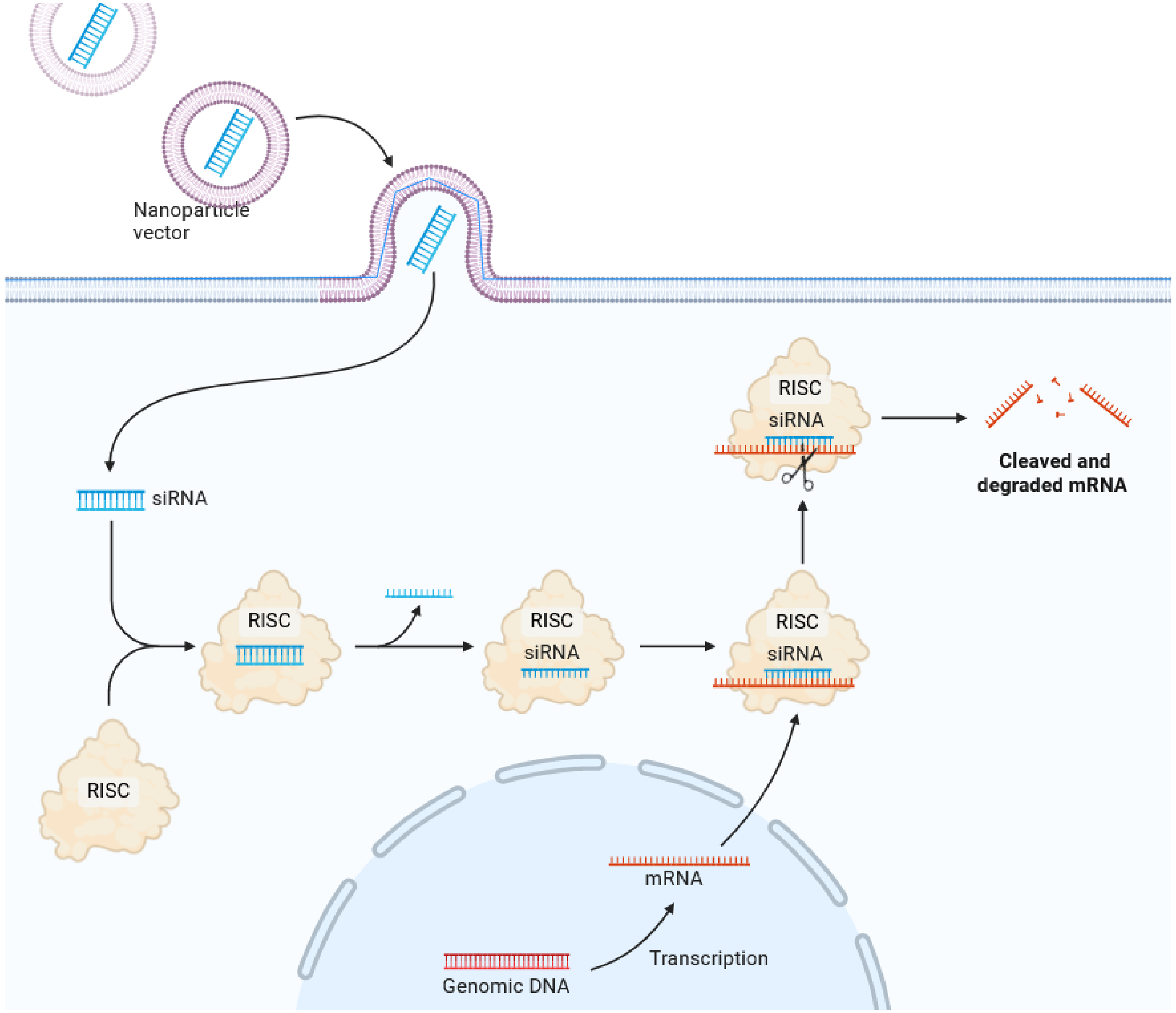


How to identify receptors?

siRNA:

20–30 nt noncoding RNAs

physiological process to induce gene silencing

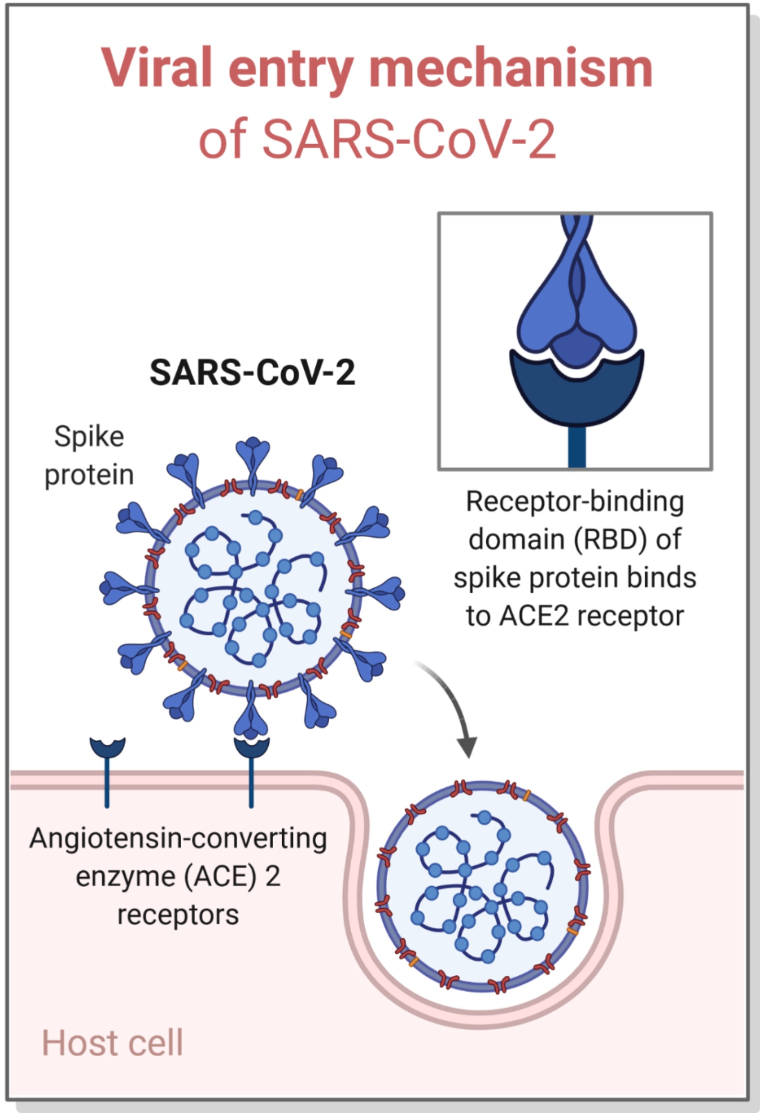


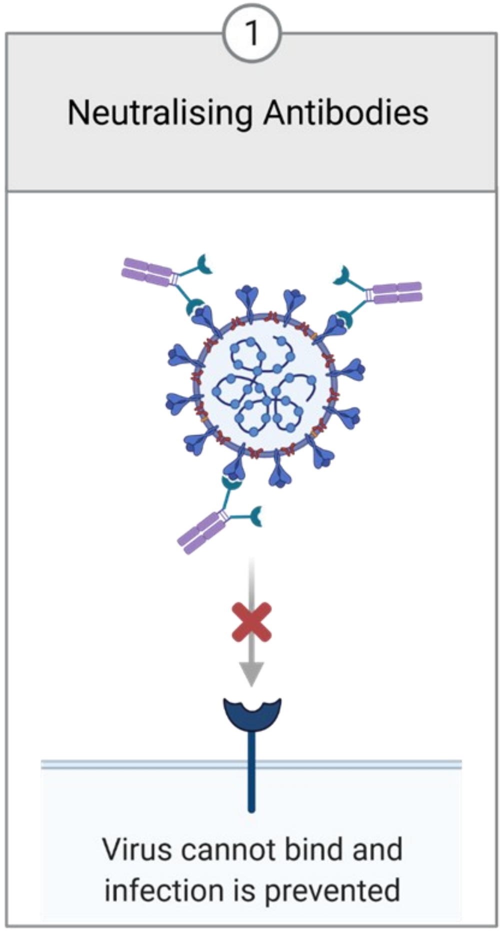
Transient regulation of protein expression

Sequence-specific knockdown of gene function

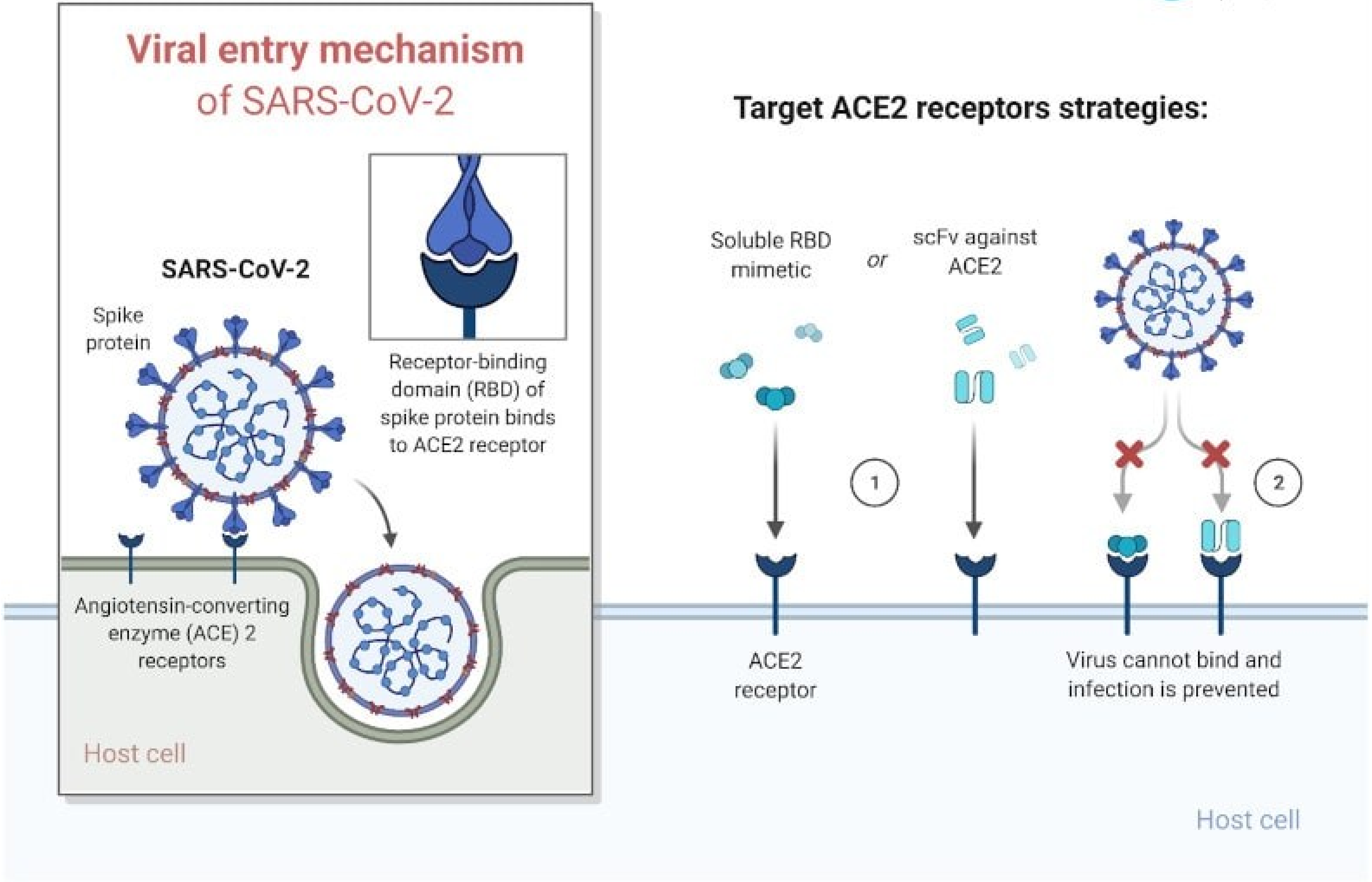
RNA-induced silencing complex (RISC)

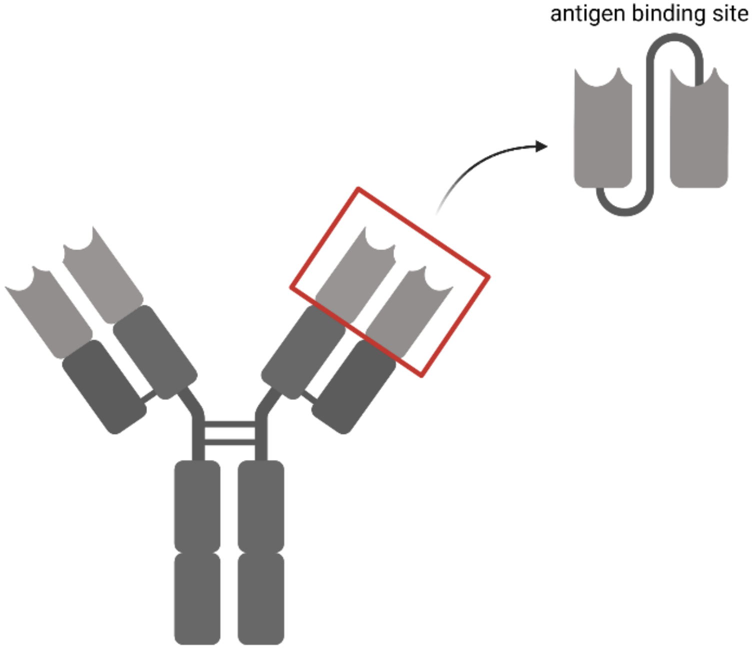
Therapeutic strategies to prevent virus entry





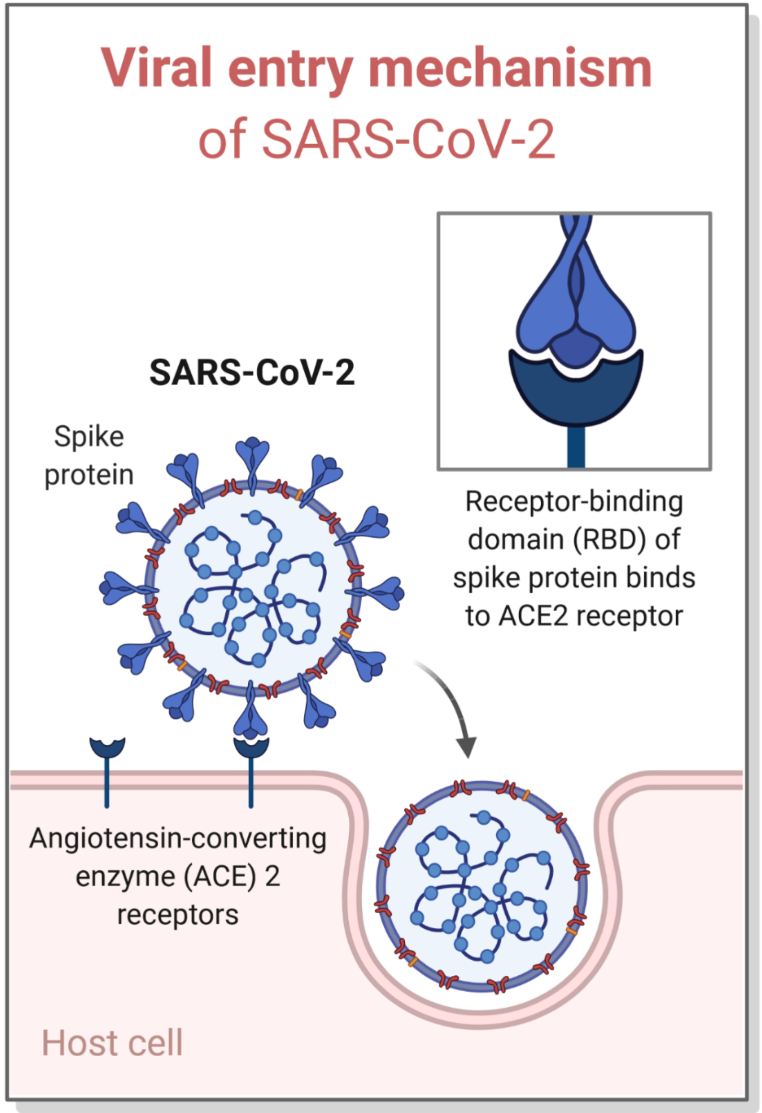
Therapeutic strategies to prevent virus entry

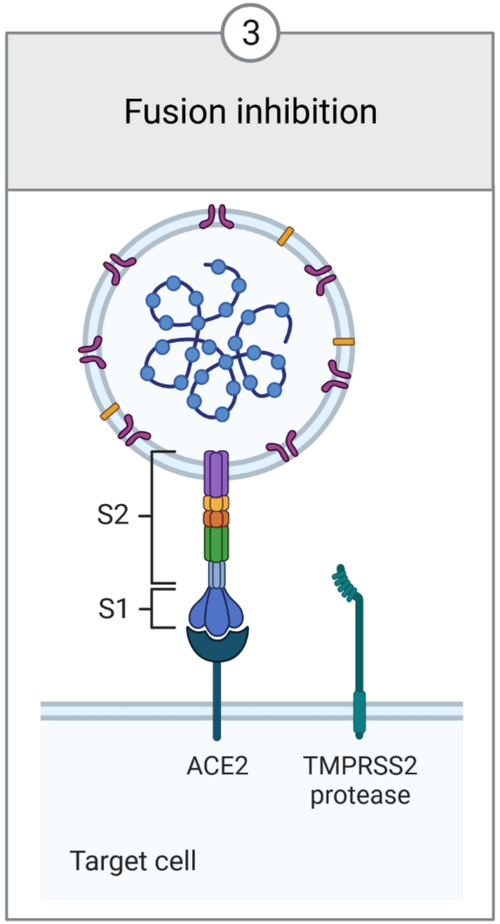




Single chain variable fragment (scFv)

Therapeutic strategies to prevent virus entry



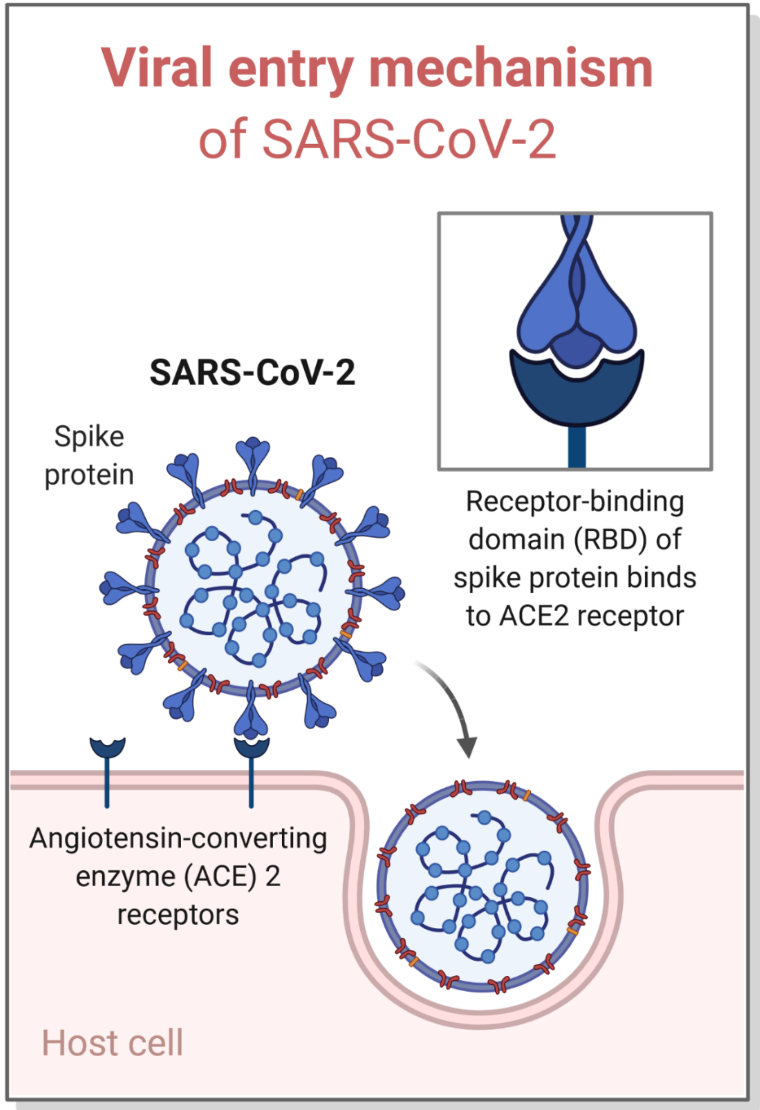


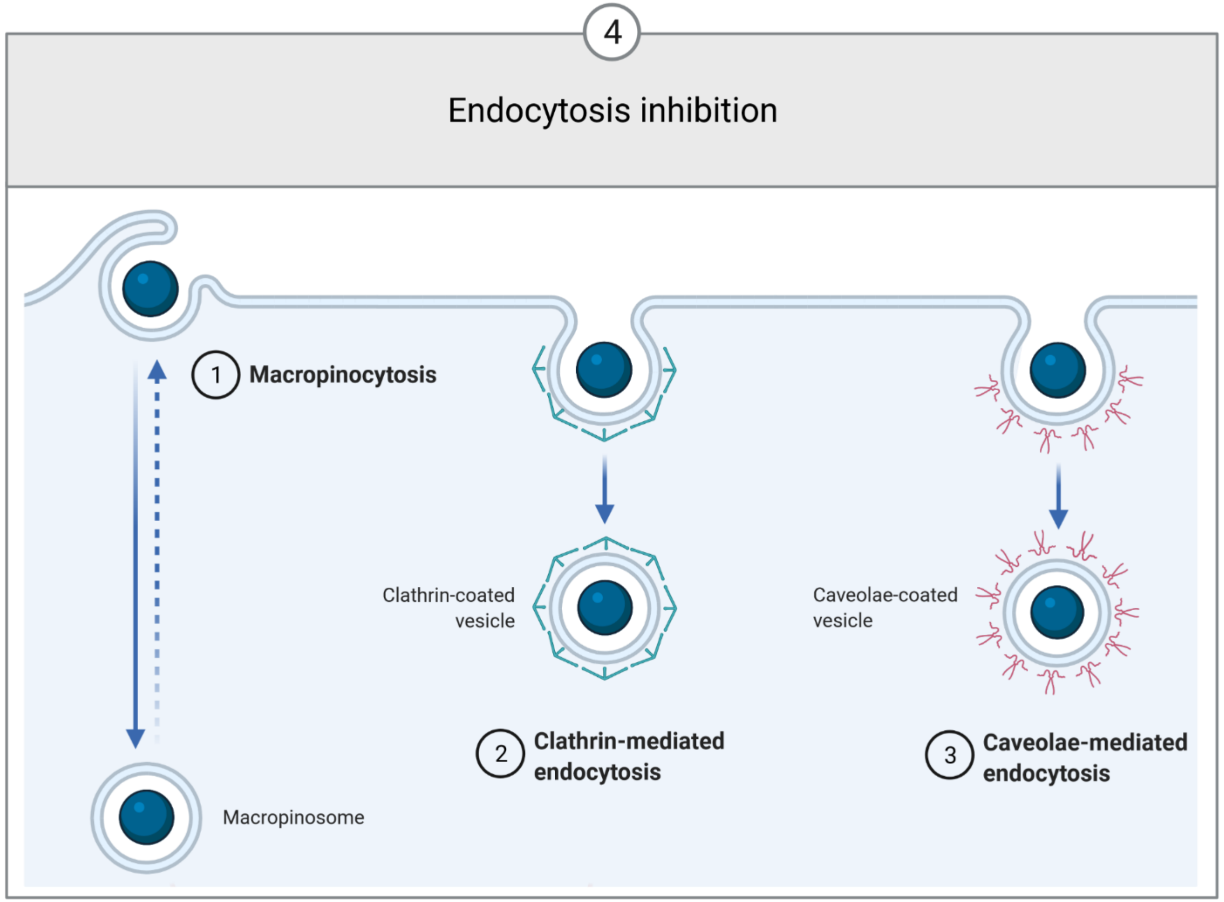
block conformational changes of glycoprotein necessary to induce fusion

or

regulate protease activity

Therapeutic strategies to prevent virus entry





BUT: not every solution is applicable *in vivo*

Thank you for your attention!

Questions?