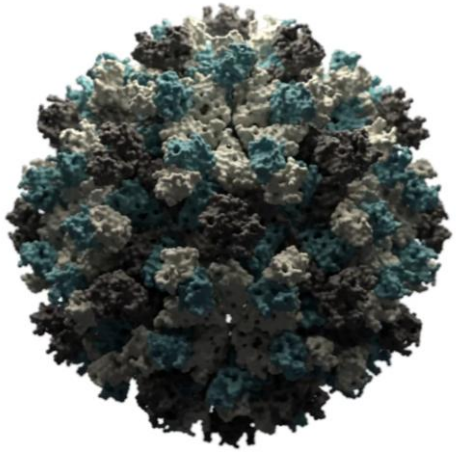


Assembly, Egress and Maturation of Viruses

Department for Molecular and Medical Virology

Dr. Birthe Ehlert
Working group of Prof. Eike Steinmann
Medical Faculty
Ruhr-University Bochum

https://www.ruhr-uni-bochum.de/virologie

The „ins and outs“ of a virus

Entry

Trafficking to replication site

Gene expression, genome replication

Trafficking of viral proteins and RNA to assembly sites

Assembly

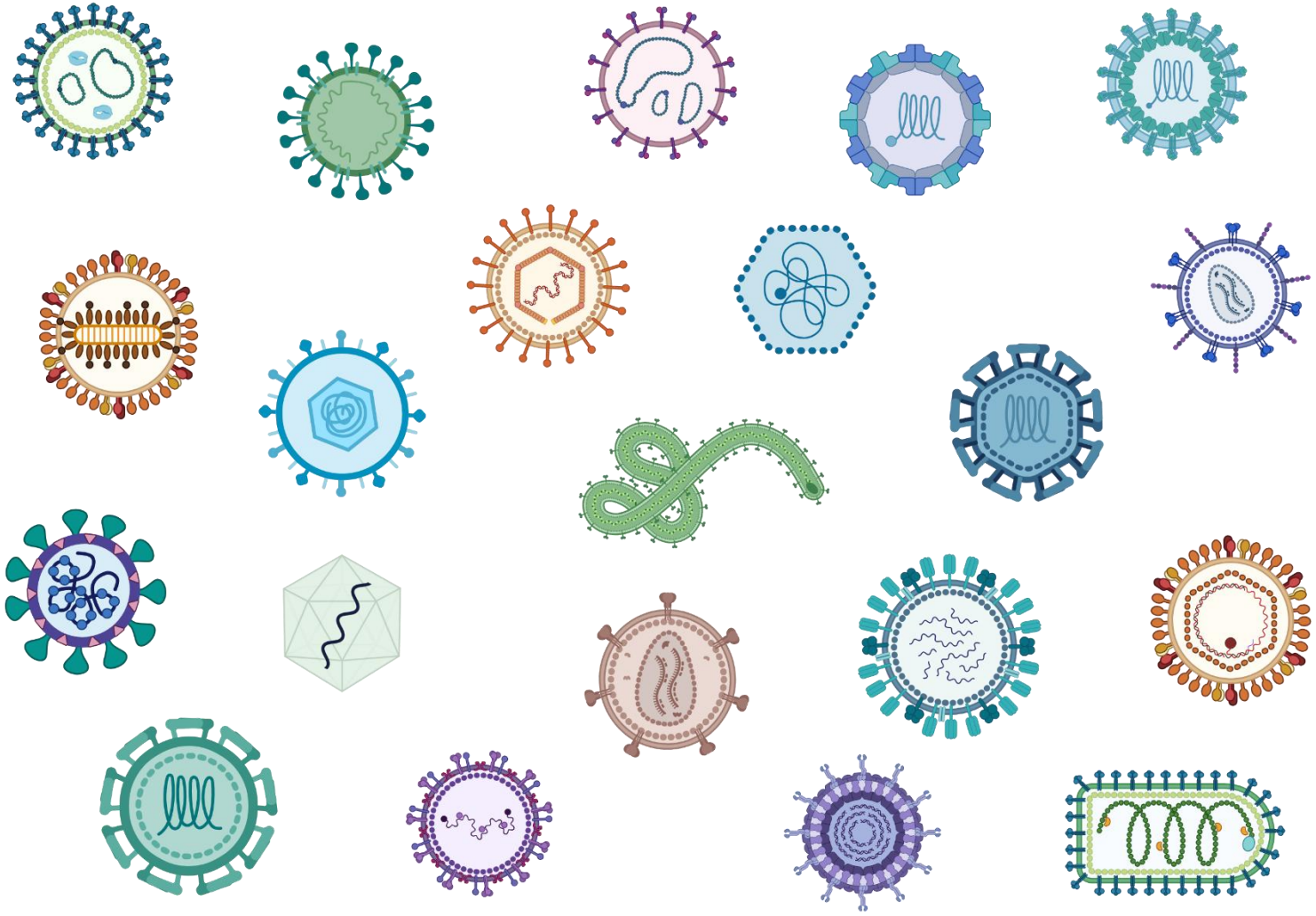
Exit/Egress

Maturation

Damage done

Todays lecture

Structure of viral particle determines how and where it is formed



Requirements for directed trafficking of viral proteins and nucleid acids

Where in the cell do viruses assemble and get released?

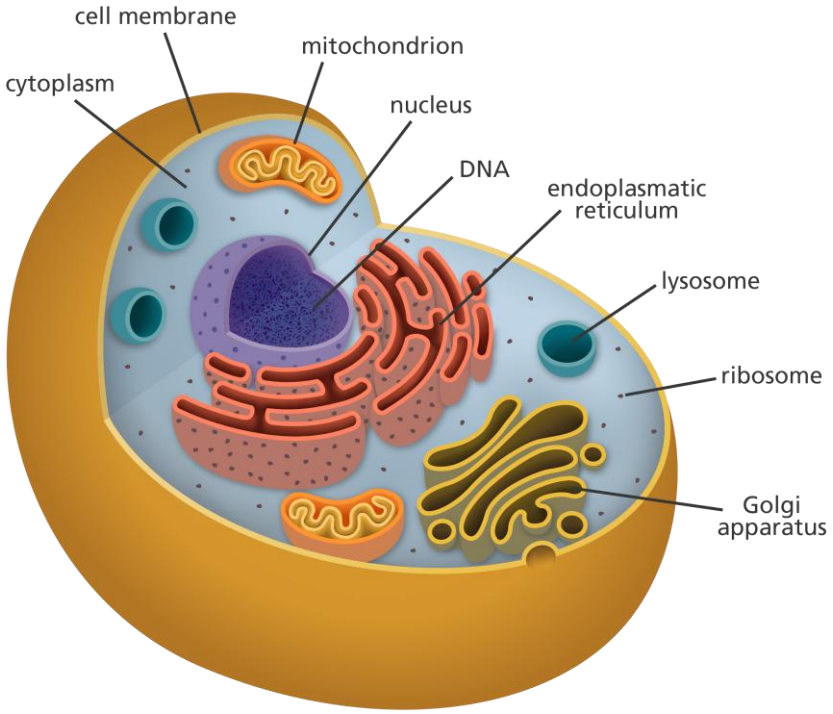


Table 12.1 Intracellular trafficking requirements for virus assembly

Assembly site(s)	Viruses
Within the nucleus	Adenovirus, polyomavirus
Within the cytoplasm	Picornavirus
At the plasma membrane	Alphavirus, retrovirus, rhabdovirus
At an internal cellular membrane	Bunyavirus, coronavirus, poxvirus
Within the nucleus and at a cellular membrane	Herpesvirus, orthomyxovirus

The „ins and outs“ of a virus

Entry

Trafficking to replication site

Gene expression, genome replication

Trafficking of viral proteins and RNA to assembly sites

Assembly

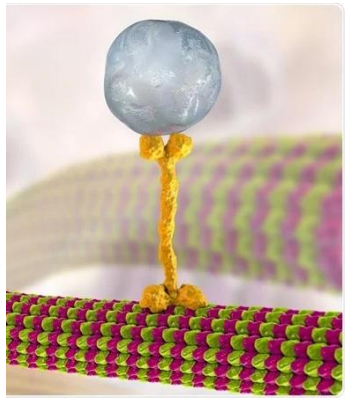
Exit/Egress

Maturation

Damage done

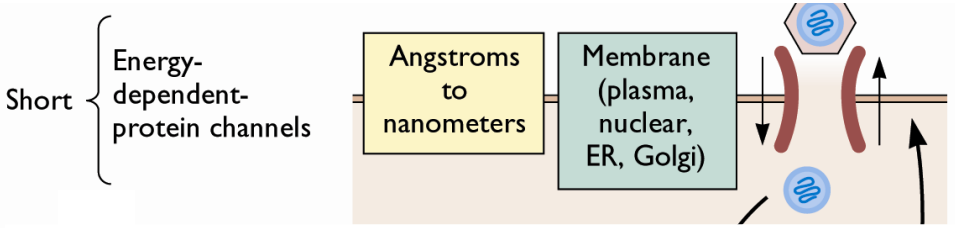
Directional movement

- **Short range**: Crossing a membrane, release from a capsid: channels
- **Long range**: Movement on cytoskeletal tracks
  - **Actin**: Myosin motor proteins
  - **Microtubles**: dynein and kinesin motor proteins



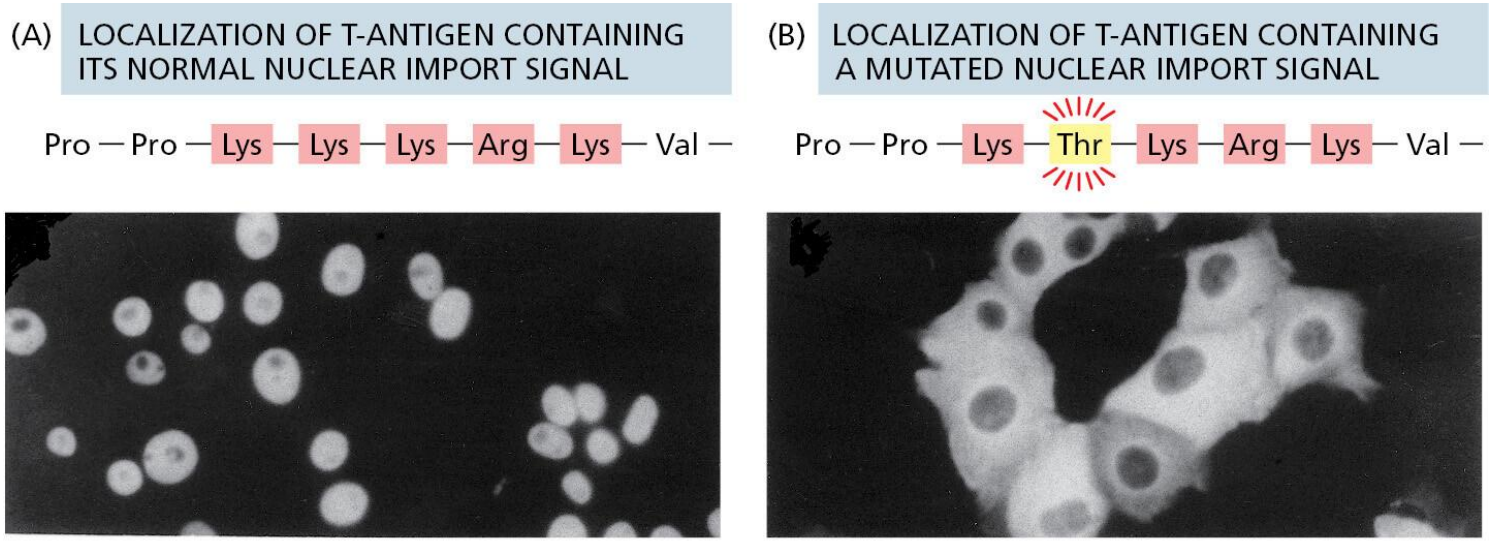
Kateryna Kon/Shutterstock.com

Moving in heavy traffic



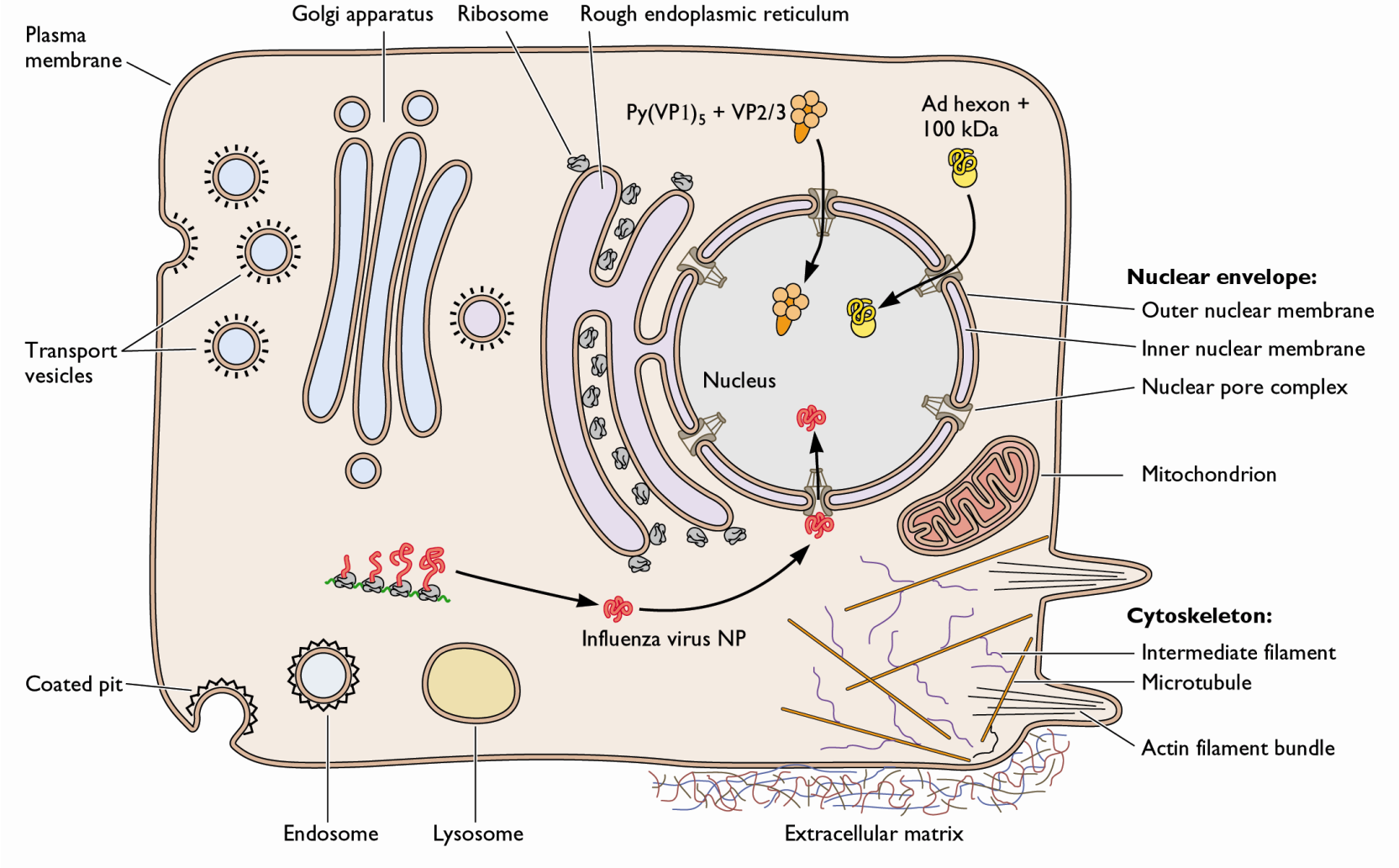
Viral proteins have „addresses“

- **Membrane targeting**: Signal sequences, fatty acid modifications — e.g. Influenza virus HA (signal peptide), HIV Gag (myristoylation)
- **Membrane retention signals** — e.g. HCV E1 and E2 glycoproteins
- **Nuclear localization sequences (NLS):** direct proteins in nucleus — e.g. Influenza virus NP, SV40 T-Antigen
- **Nuclear export signals:** proteins shuttle into and out of nucleus — e.g. Influenza virus M1

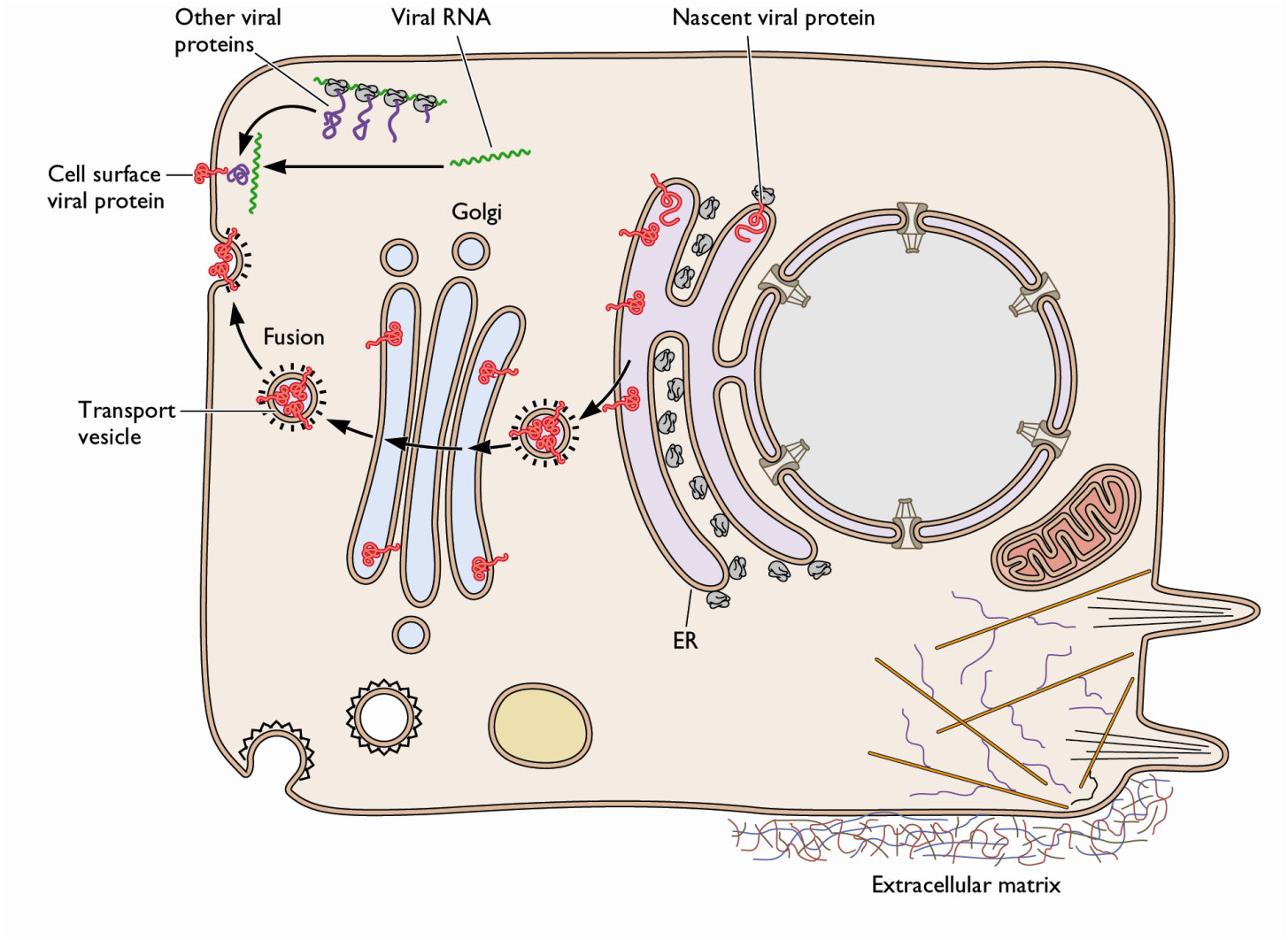


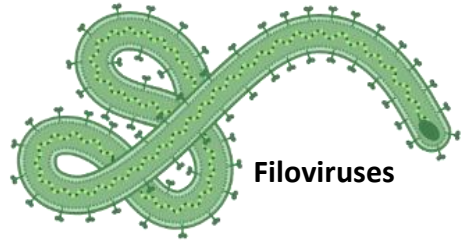
https://nerd.wwnorton.com/ebooks/epub/mboc7/EPUB/content/4.3.5-chapter12.xhtml

Localization of viral proteins to nucleus

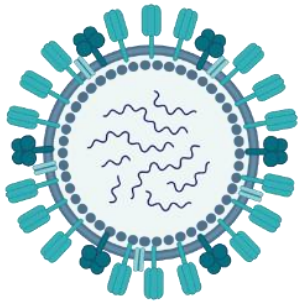


Localization of viral proteins to the plasma membrane

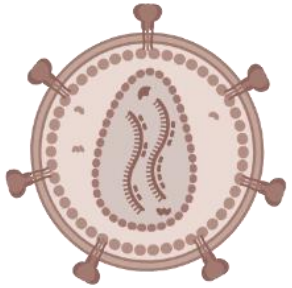




Filoviruses

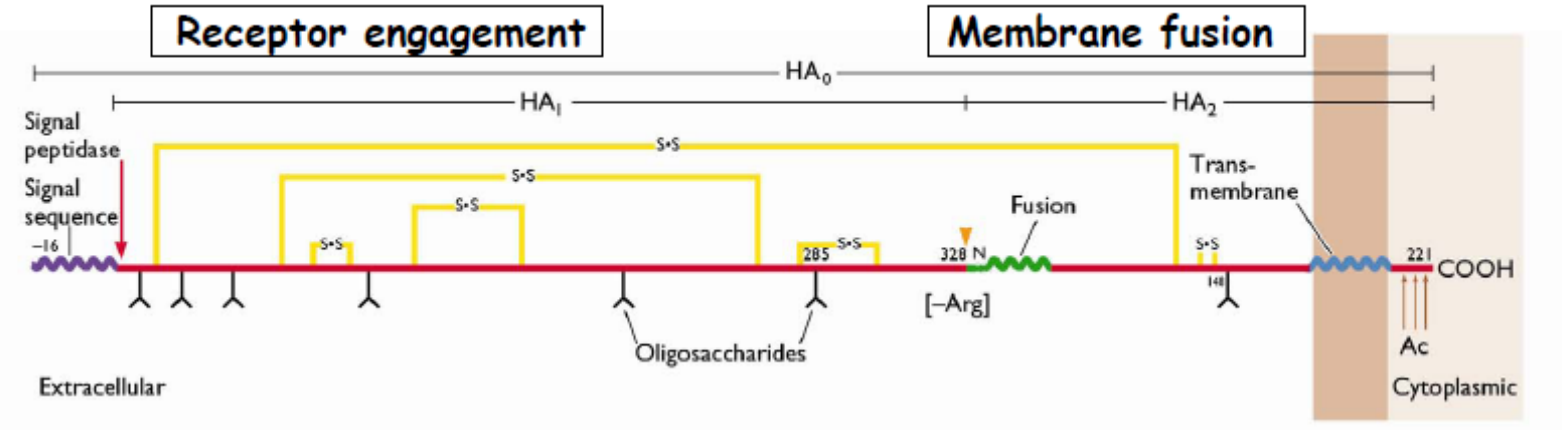


Influenza virus



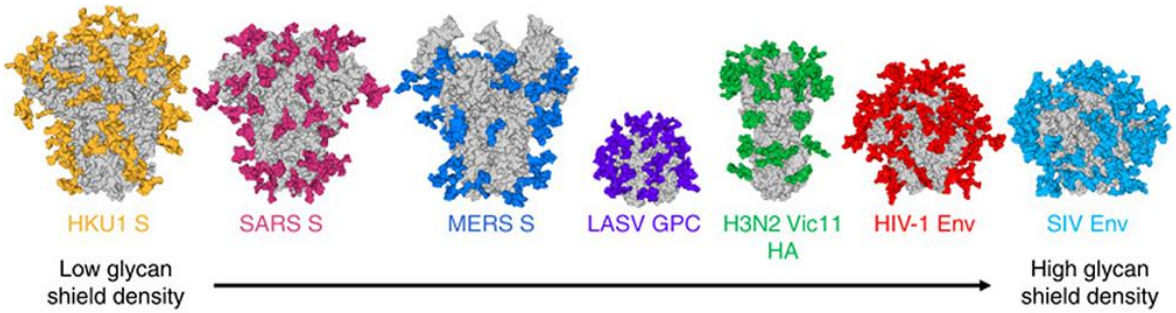
Retrovirus

Assembly at the plasma membrane: transport of viral glycoproteins

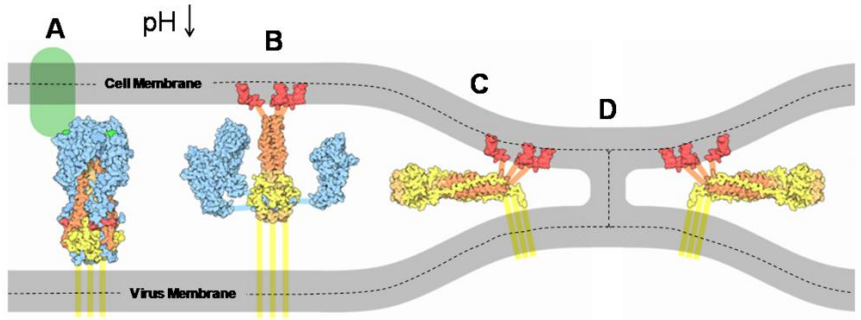


Signal peptide | **Glycans** | **Disulfide bonds** | **Cleavage sites** | **Membrane anchor**

ER import | **Folding Immune protection** | **Folding** | **Activation** | **Complete Membrane fusion**



https://www.dovepress.com/glycan-nanostructures-of-human-coronaviruses-peer-reviewed-fulltext-article-IJN



Hamilton et al., 2012, Viruses

Biogenesis of viral glycoproteins in the secretory pathway

ER import

Glycosylation, folding, disulfide bonds, oligomerization

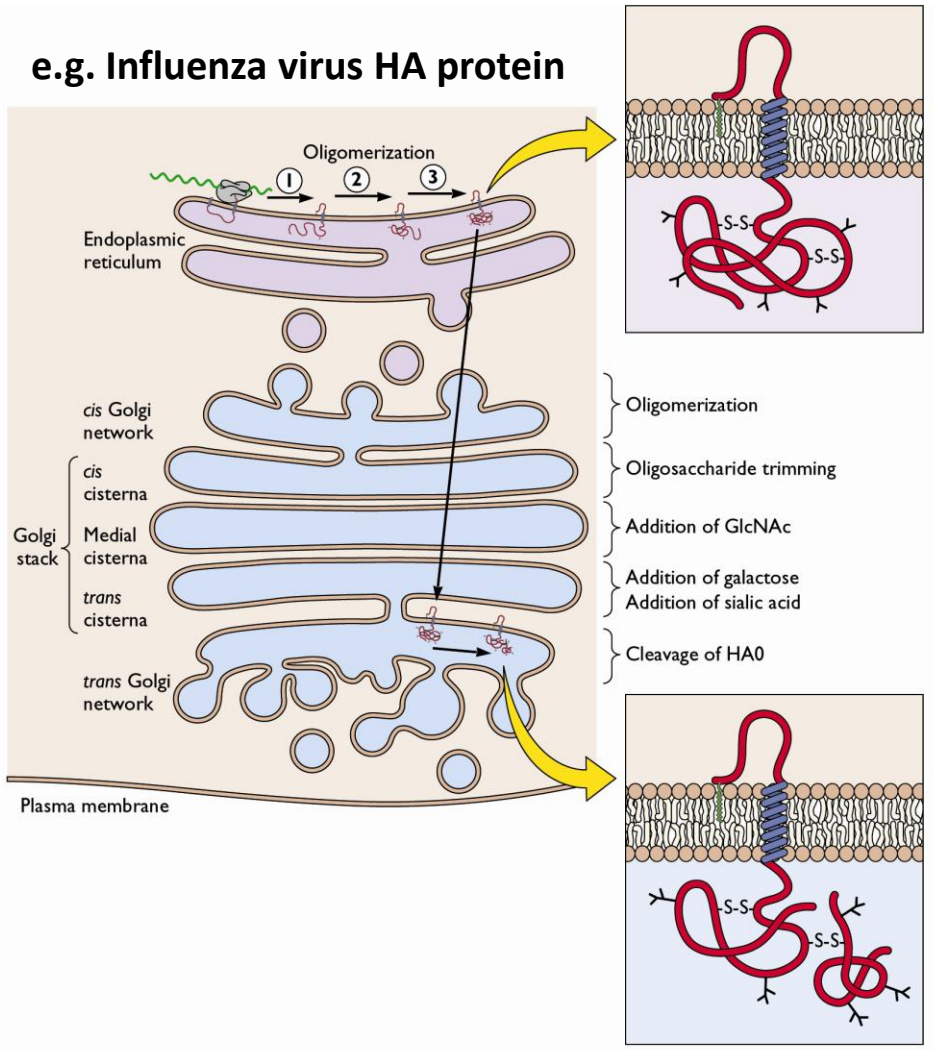
Transport into Golgi apparatus

Trimming of glycans

Cleavage

Transport to the plasma membrane

e.g. Influenza virus HA protein



Targeting of a nascent protein to the ER membrane

ER import

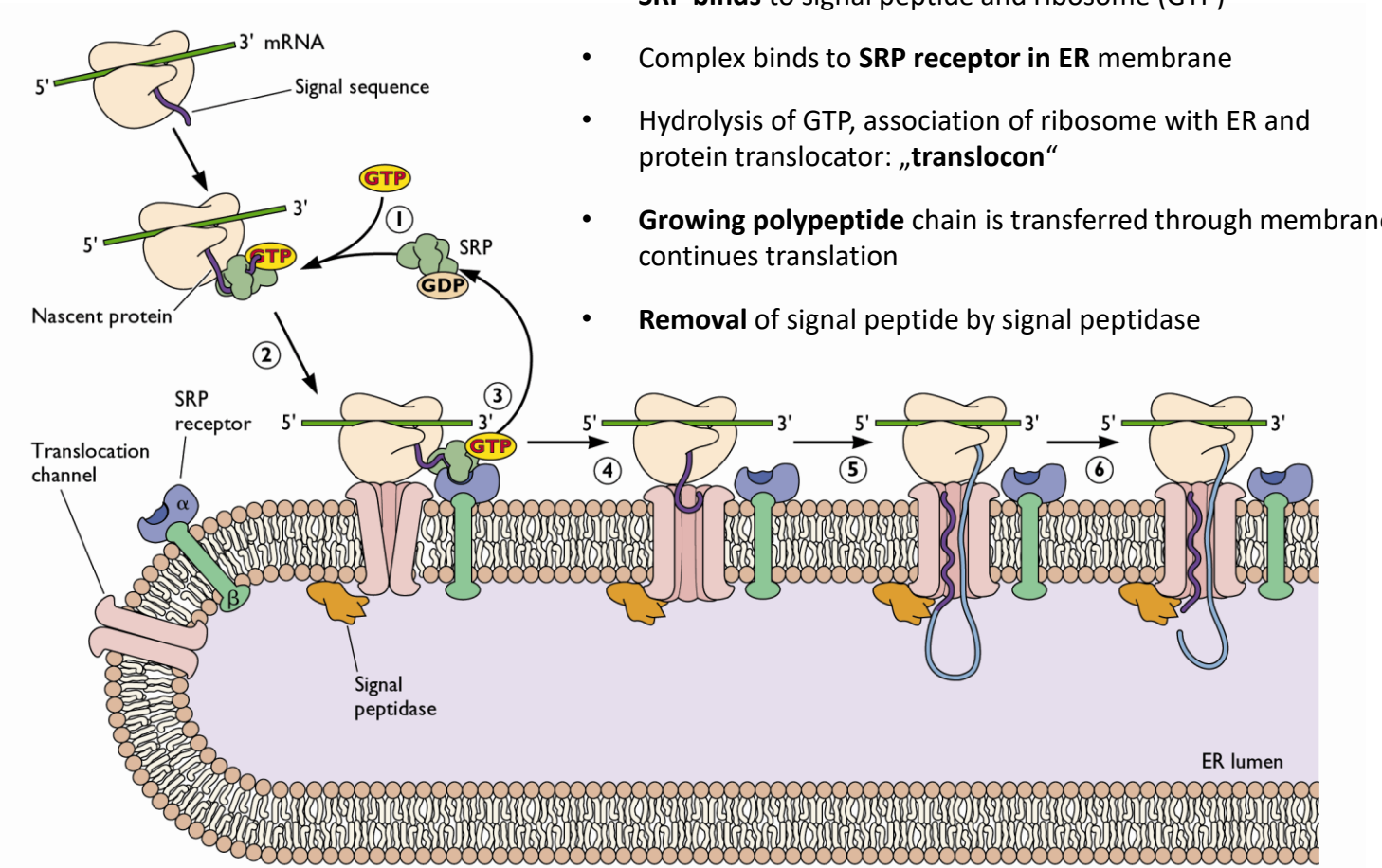
Glycosylation, folding, disulfide bonds, oligomerization

Transport into Golgi apparatus

Trimming of glycans

Cleavage

Transport to the plasma membrane



- mRNA encoding a protein with **signal peptide for ER**
- **SRP binds** to signal peptide and ribosome (GTP)
- Complex binds to **SRP receptor in ER** membrane
- Hydrolysis of GTP, association of ribosome with ER and protein translocator: „**translocon**“
- **Growing polypeptide** chain is transferred through membrane, continues translation
- **Removal** of signal peptide by signal peptidase

SRP = signal recognition particle

Structure of N-linked oligosaccharides

ER import

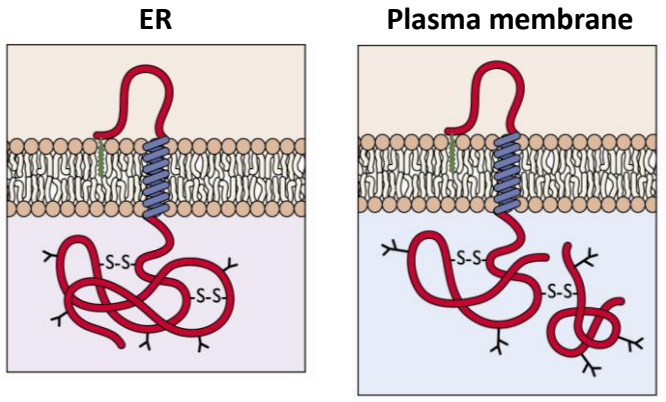
Glycosylation, folding, disulfide bonds, oligomerization

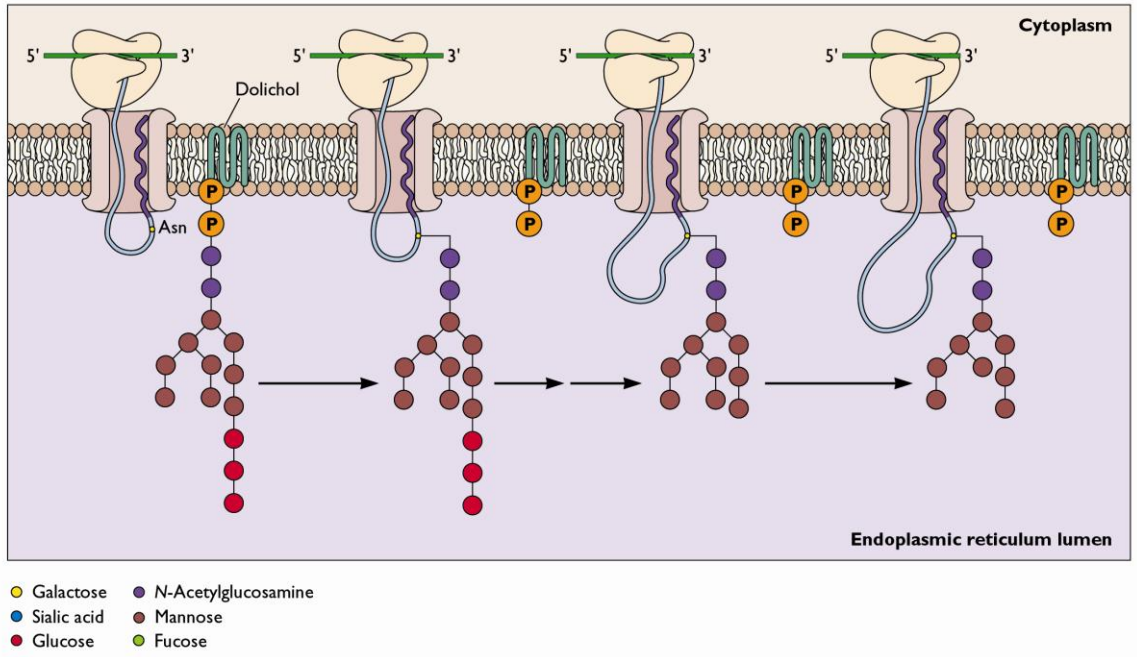
Transport into Golgi apparatus

Trimming of glycans

Cleavage

Transport to the plasma membrane





- **Mannose-rich oligosaccharide added** via N-glycosidic bond to **asparagine residue**
- **Sugar precursor** is transferred to N-linked glycosylation sites as **protein translocates into the ER**
- **Three glucose residues and one mannose trimmed** and additional sugars are added when protein travels through **golgi**

Avian Influenza viruses: HA cleavage determines pathogenicity

ER import

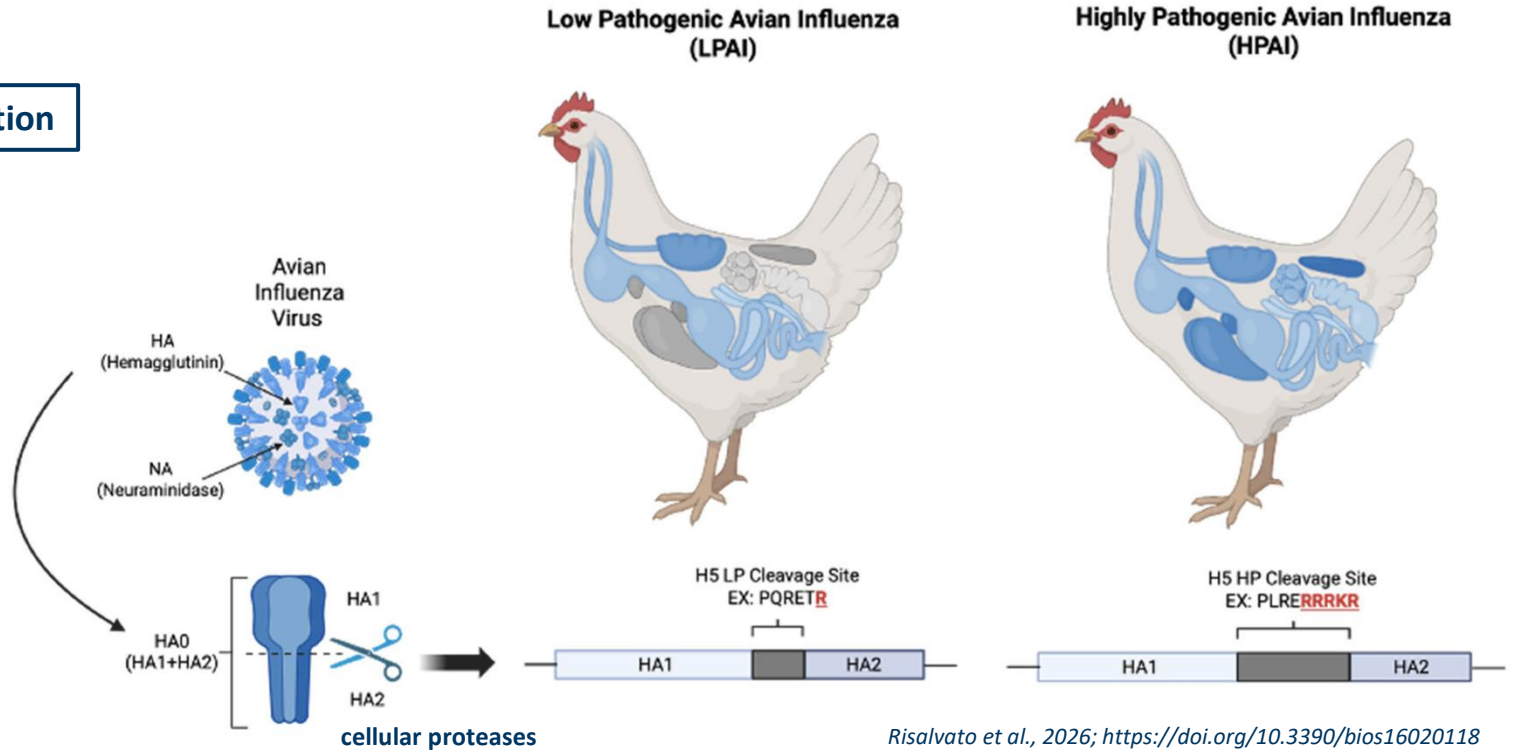
Glycosylation, folding, disulfide bonds, oligomerization

Transport into Golgi apparatus

Trimming of glycans

Cleavage

Transport to the plasma membrane



Risalvato et al., 2026; https://doi.org/10.3390/bios16020118

Compartments in the secretory pathway

ER import

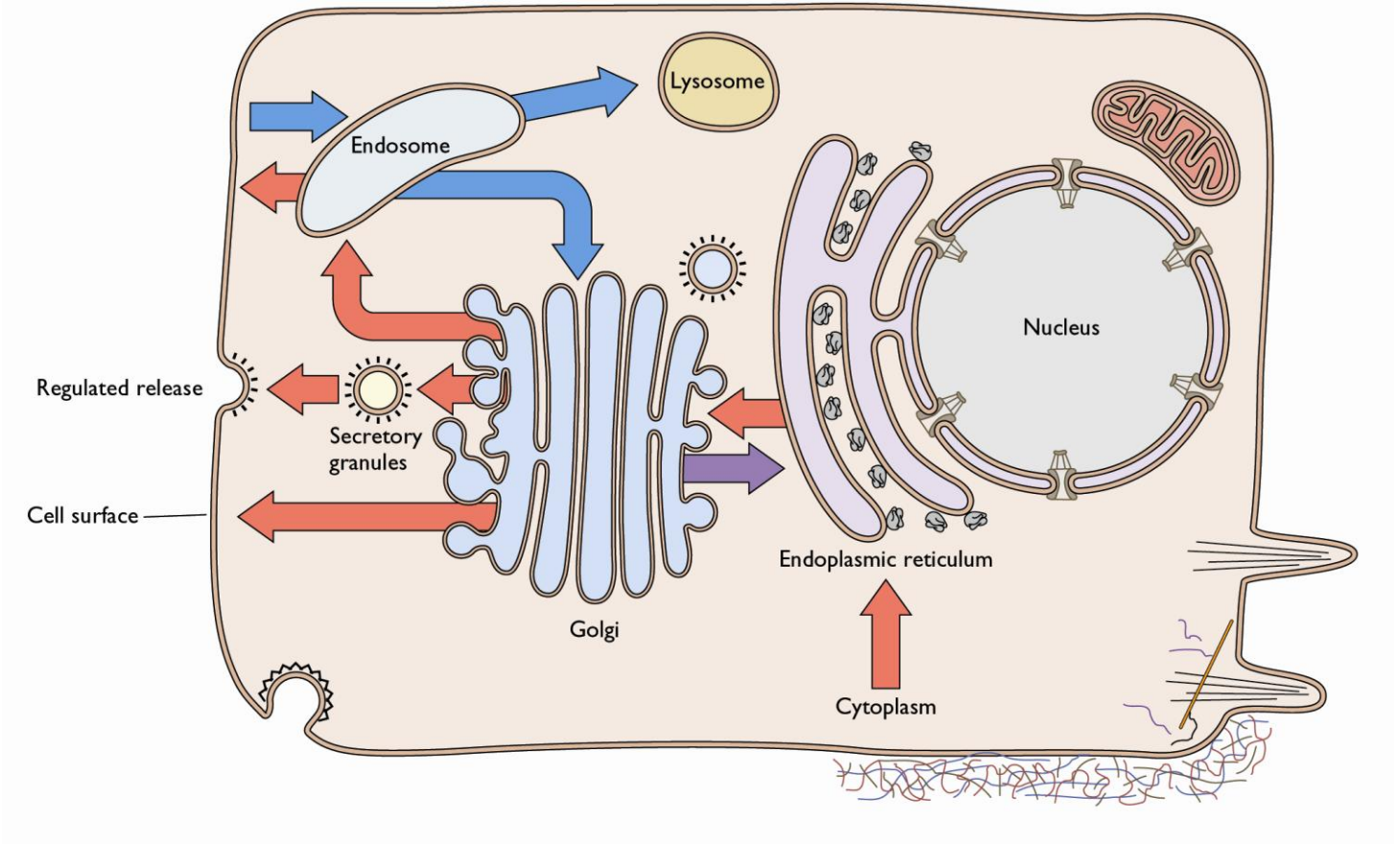
Glycosylation, folding, disulfide bonds, oligomerization

Transport into Golgi apparatus

Trimming of glycans

Cleavage

Transport to the plasma membrane



Protein transport from the ER to the Golgi

ER import

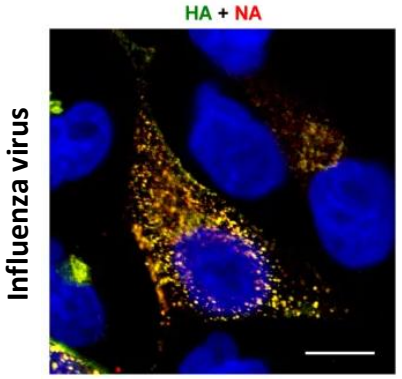
Glycosylation, folding, disulfide bonds, oligomerization

Transport into Golgi apparatus

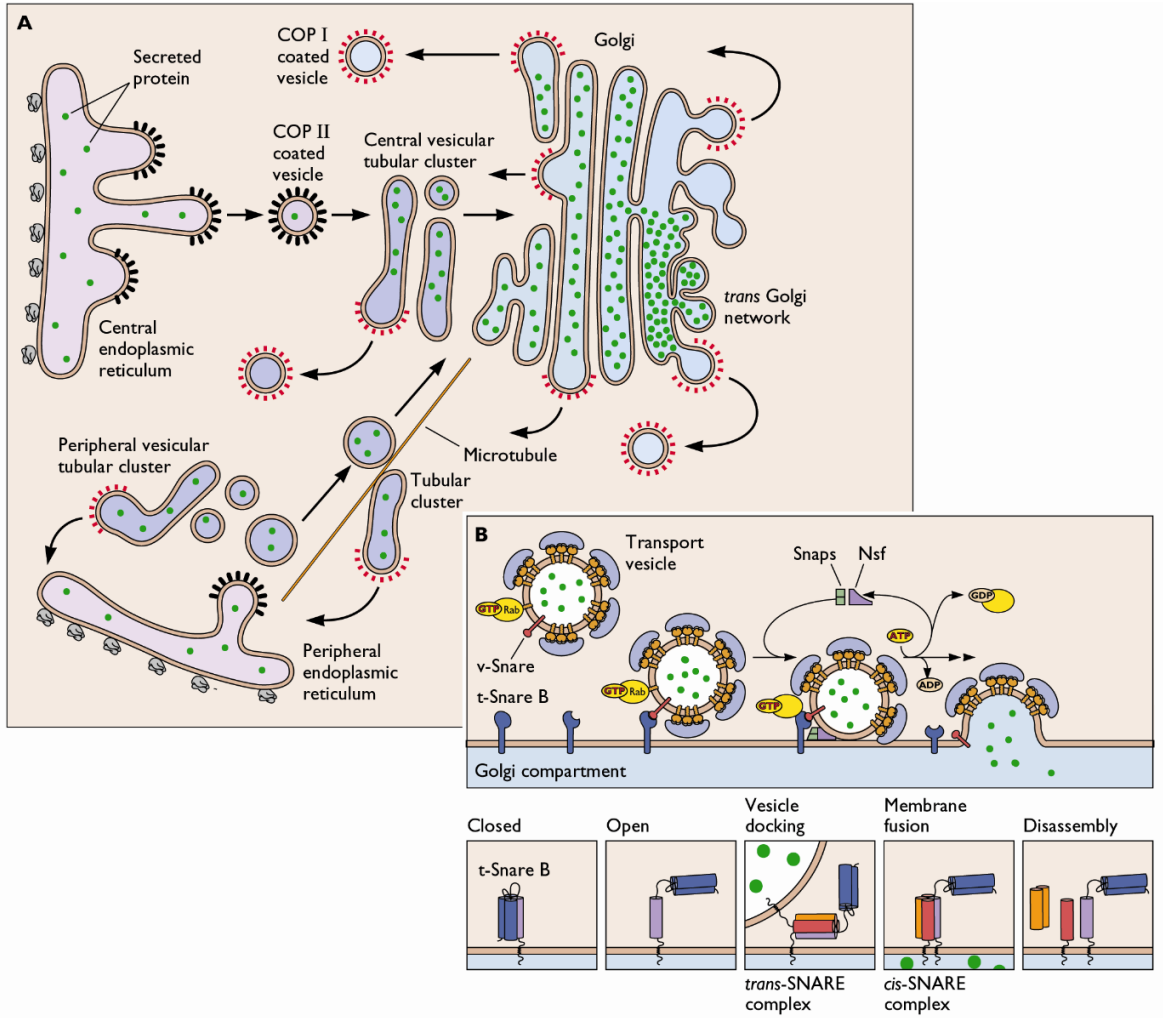
Trimming of glycans

Cleavage

Transport to the plasma membrane



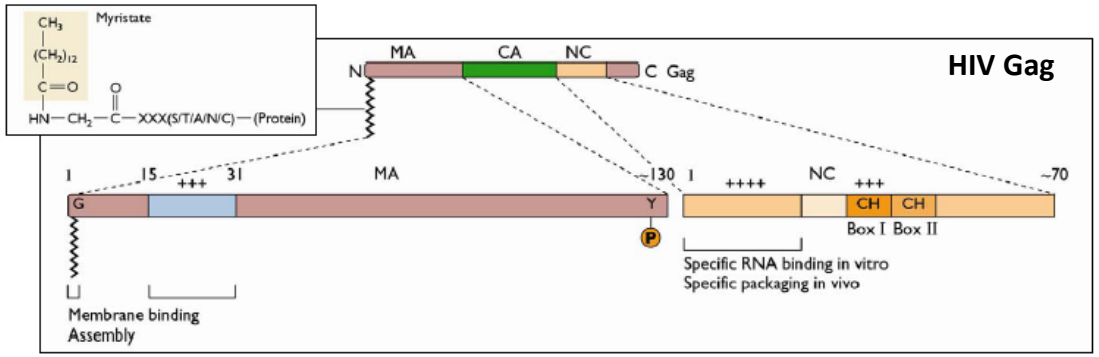
Sato et al., 2019

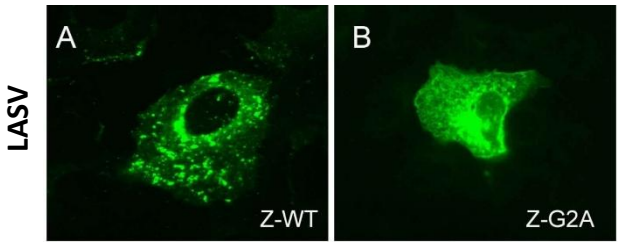


Other means of directed transport of viral proteins

Lipid-Plus-Protein-Signals (SP-independent)

- **Myristoylation:** addition of 14-carbon, saturated fatty acid myristate to **N-terminal glycine**
- Essential for **membrane association** of HIV Gag and LASV Z and therefore **viral budding**
- Myristoylation of VP4 is essential for poliovirus **assembly**

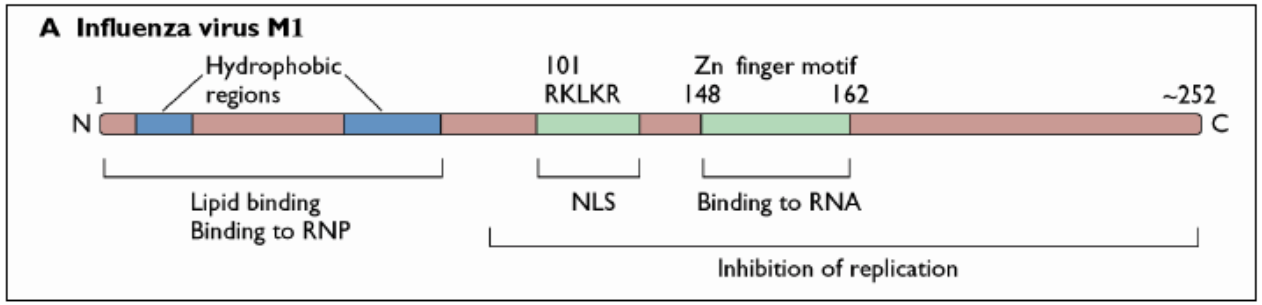




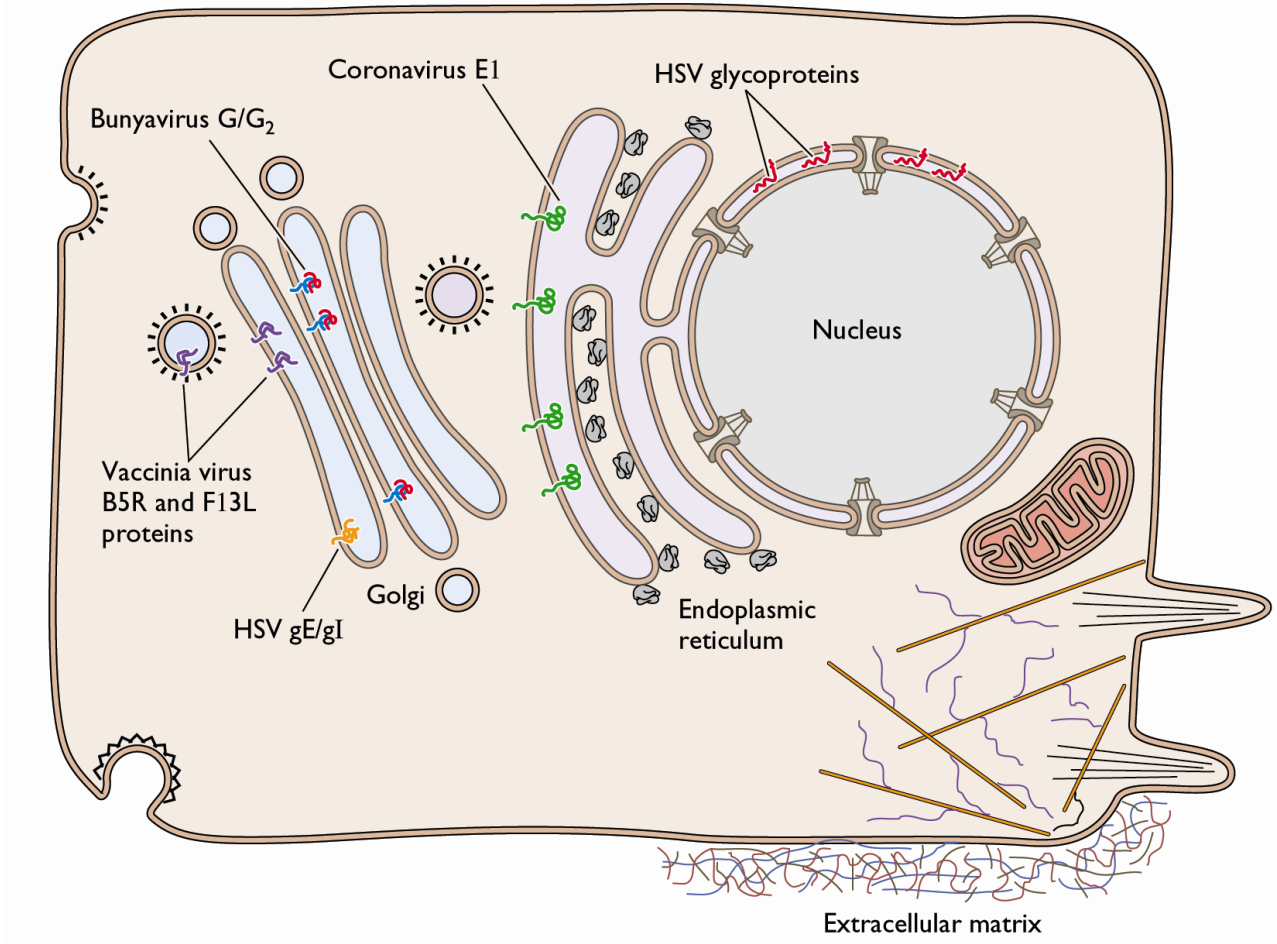
Strecker et al., 2006

Protein-Signals (SP-independent)

- **Matrix protein of negative strand RNA viruses:** Essential for genome packaging
- No lipid modification, **membrane association** intrinsic property of the protein
- Membrane binding maybe enhanced by interaction with viral membrane proteins

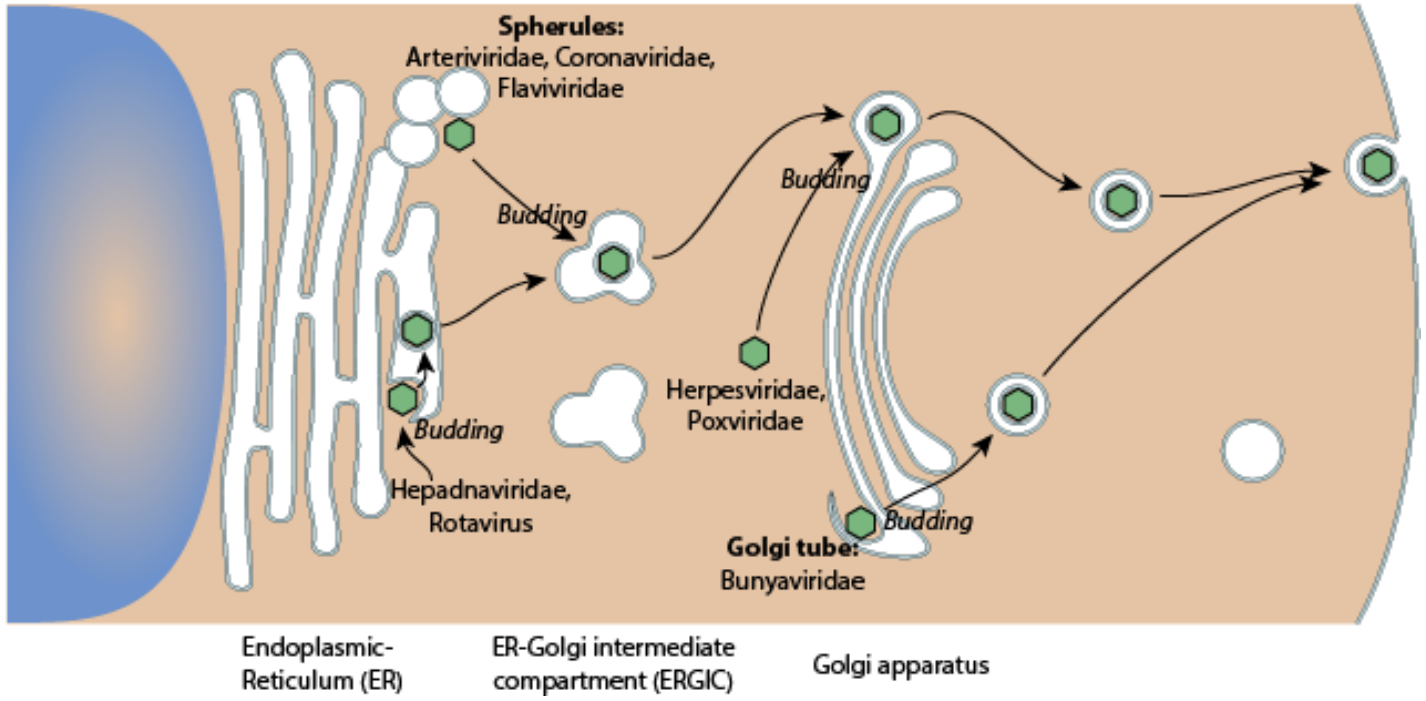


Site of budding depends on localization of envelope proteins



Budding at ER, ERGIC or Golgi apparatus indicates **viral particles** are **exported by cellular exocytosis**

Site of budding correlates with site of genome replication



Correlation between site of **budding** and site of **replication**

- **Enveloped (+) RNA viruses and dsRNA viruses:** bud on ER or in the ERGIC (replication in close proximity)
- **Bunyaviruses:** bud at Golgi (replicate in Golgi membrane invaginations)

The „ins and outs“ of a virus

Entry

Trafficking to replication site

Gene expression, genome replication

Trafficking of viral proteins and RNA to assembly sites

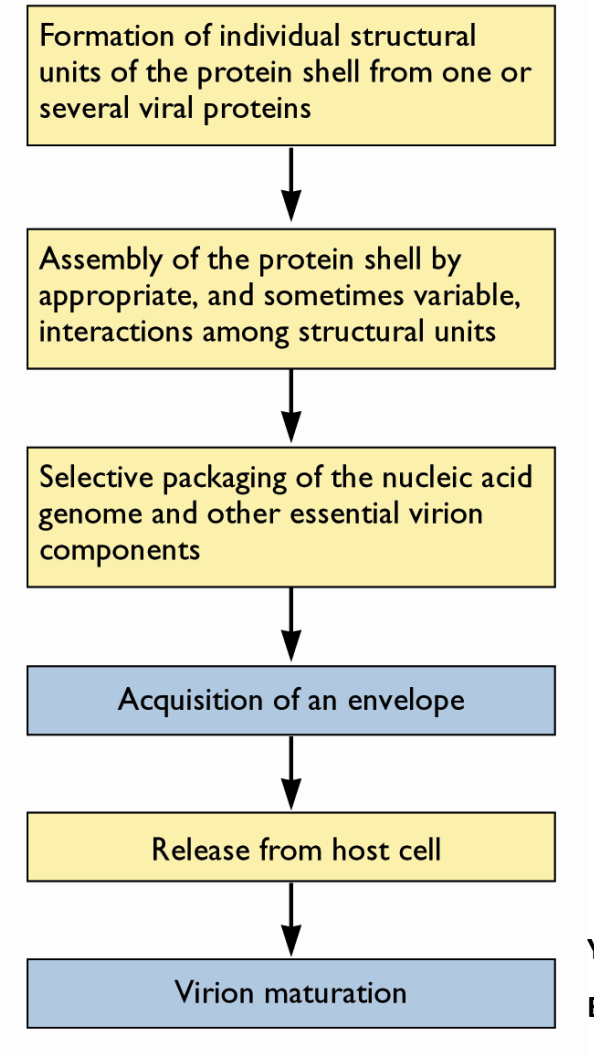
Assembly

Exit/Egress

Maturation

Damage done

Hypothetical pathway of virion assembly and release

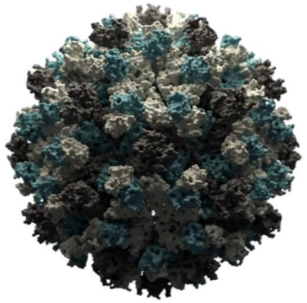


Yellow: common to all viruses

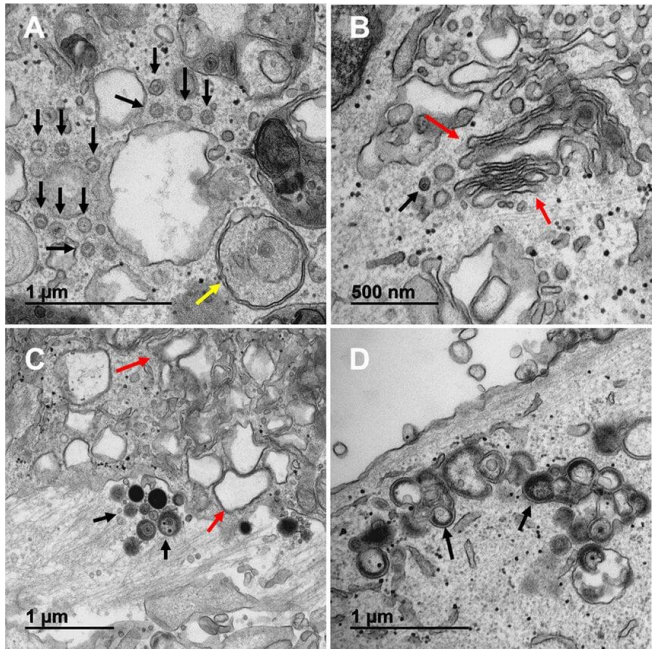
Blue: common to many viruses

Methods of studying virus assembly and egress

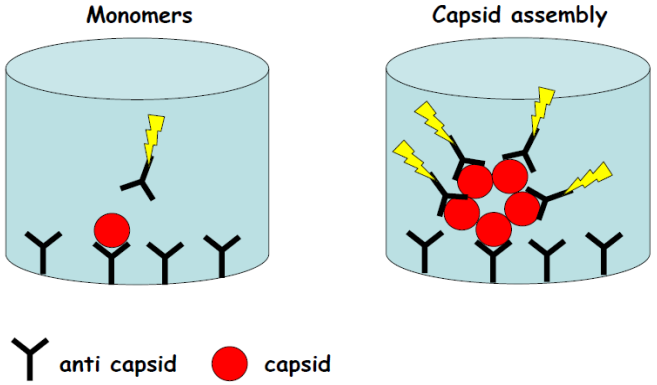
Structural studies of virus particles



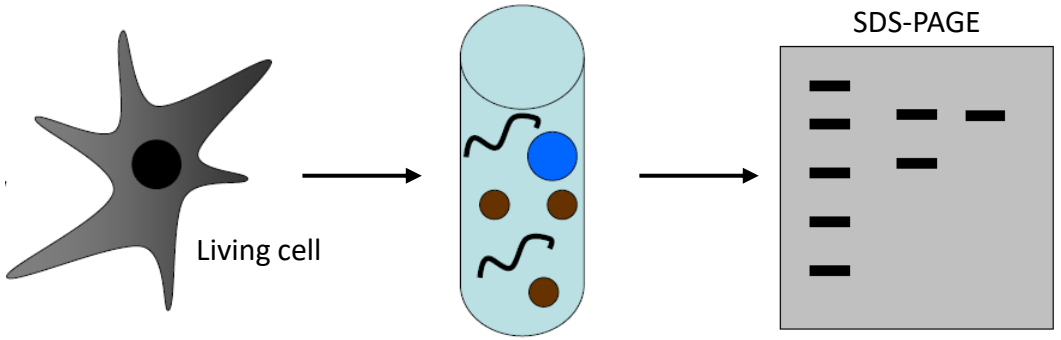
Visualization by microscopy (EM, IFM, GFP etc.)



Biochemical and genetic analysis of **assembly intermediates**



Recombinant DNA technology
(e.g. *in vitro* translation system)



ATP, GTP, amino acids,
radiolabeled cDNA,
cytoplam. extracts
Membrane vesicles

Challenges of virus assembly

- **Coordination** of subunit expression, **assembly** into structural units and **transport** to the site of capsid assembly
- Coordination of **capsid assembly** and **nucleic acid incorporation**
- Capsids: high **stability** versus efficient **disassembly** (virus entry)
- Transport of capsids to membranes harboring **envelope** proteins
- **Release** from the infected cell (budding, lysis) or direct transfer to new host cells

Virus assembly

Assemby of subunits and intermediates

Assembly of protein shells

Packaging of the viral genome

Release

Maturation

Virus assembly

Assemby of subunits and intermediates

Assembly of protein shells

Packaging of the viral genome

Release

Maturation

Three strategies for making sub-assemblies:

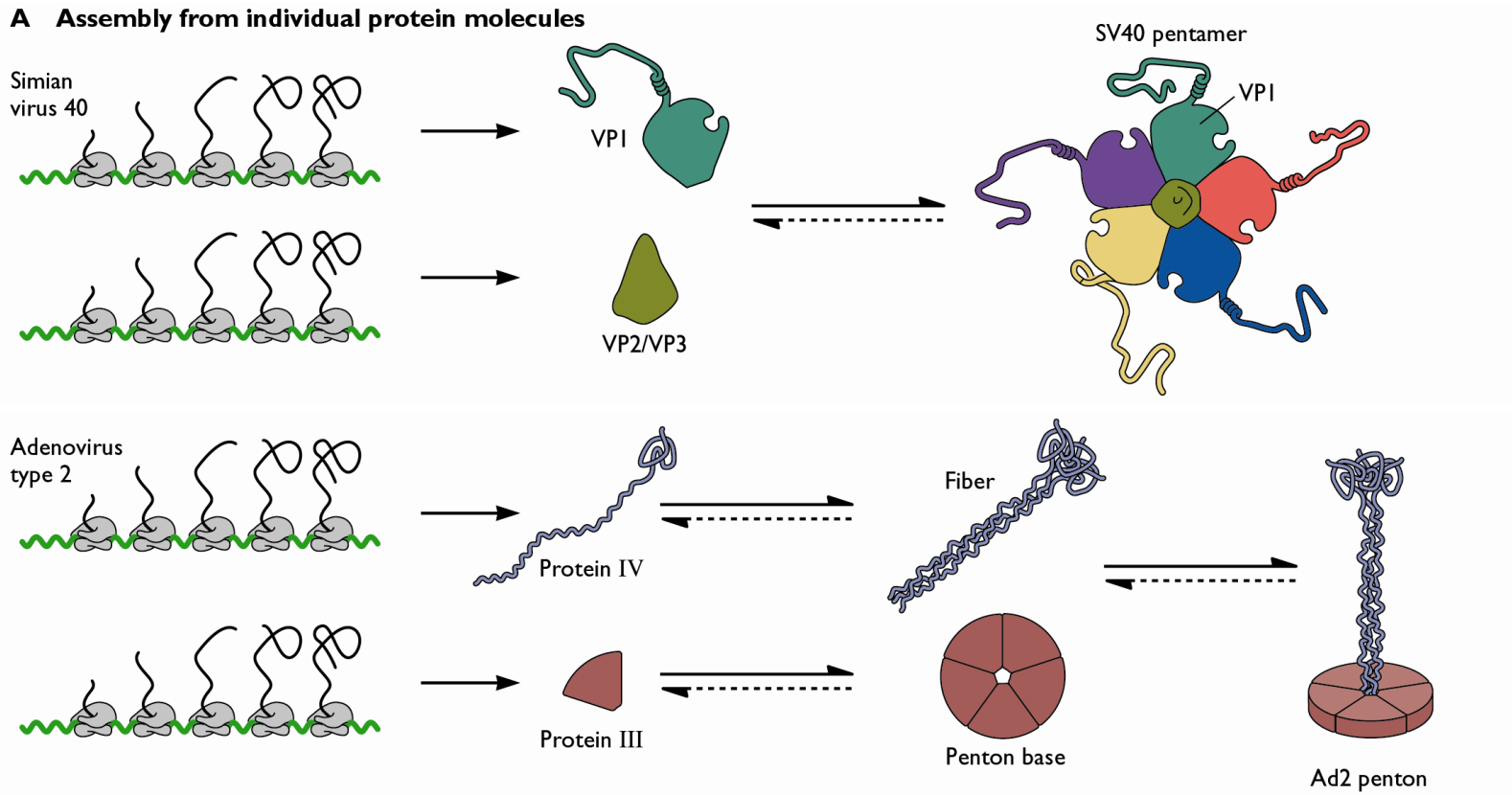
Assembly from individual protein molecules

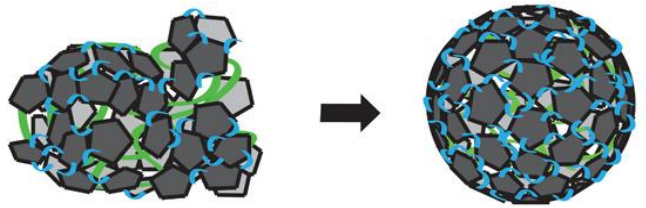
Assembly from polyprotein precursor

Chaperone-assisted assembly

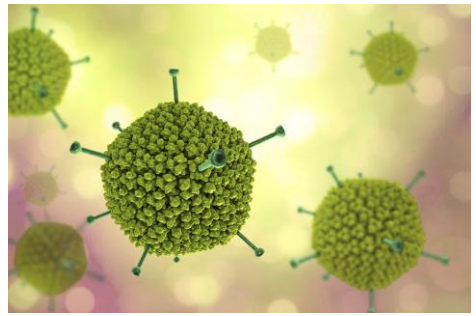
Assembly from individual protein molecules

- Subunits interlock, all **information contained within primary structure**, no chaperons and no conformational changes necessary
- Individual subunits must encounter each other: **high expression** level required



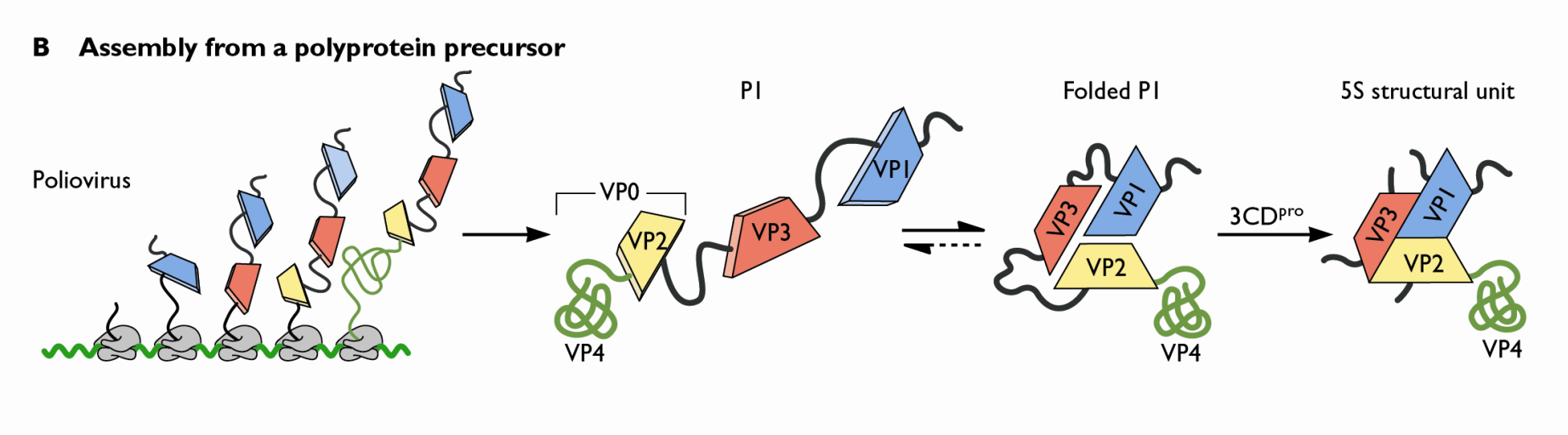


Rosmalen et al., 2020



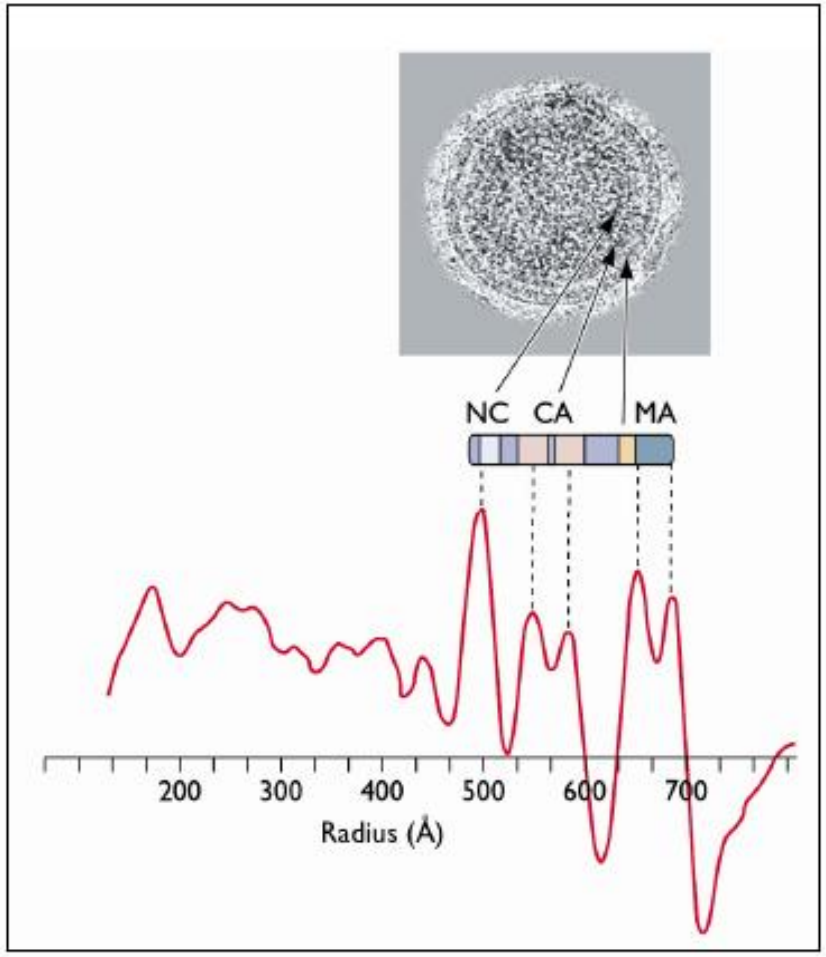
https://www.antibodiesinc.com

Assembly from polyprotein precursor: picornaviruses



- **Four proteins** form heteromeric structural unit
- **P1 immature structural unit**: VP0, VP3 and VP1
- Viral Protease $3CD^{pro}$ allows formation of **5S structural subunit**
- **VP4 remains covalently linked** to VP2 in VP0 until assembly is completed

Assembly from polyprotein: retroviruses



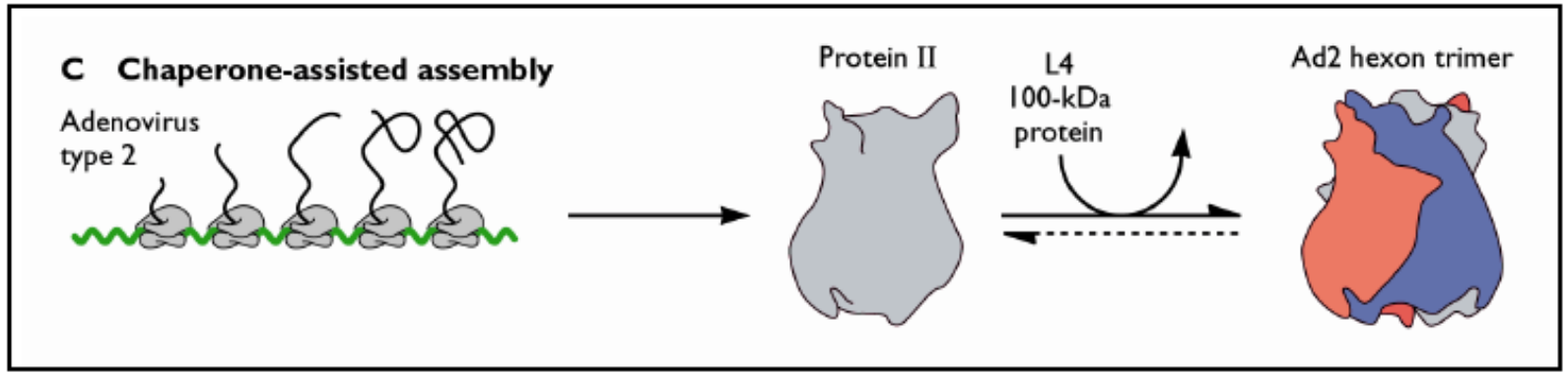
Retroviral Gag proteins:

- Polyproteins, cleaved by viral protease in budding virions: **Maturation**
- Maturation is blocked by **protease inhibitors**

Cellular and viral chaperon-assisted assembly

- **Cellular chaperons** required for folding of viral structural proteins
  - Hsp70 proteins: HIV Gag, Adeno protein IV, HBV L protein
  - Hsp68 proteins: HIV Gag
  - Chaperonin TriC: Mason-Pfizer monkey virus Gag

- **Viral chaperons**
  - Adeno 2 L4 100-kDa protein: Hexon protein
  - HSV1 VP22a: VP5



Virus assembly and release

Assemby of subunits and intermediates

Assembly of protein shells

Packaging of the viral genome

Release

Maturation

→

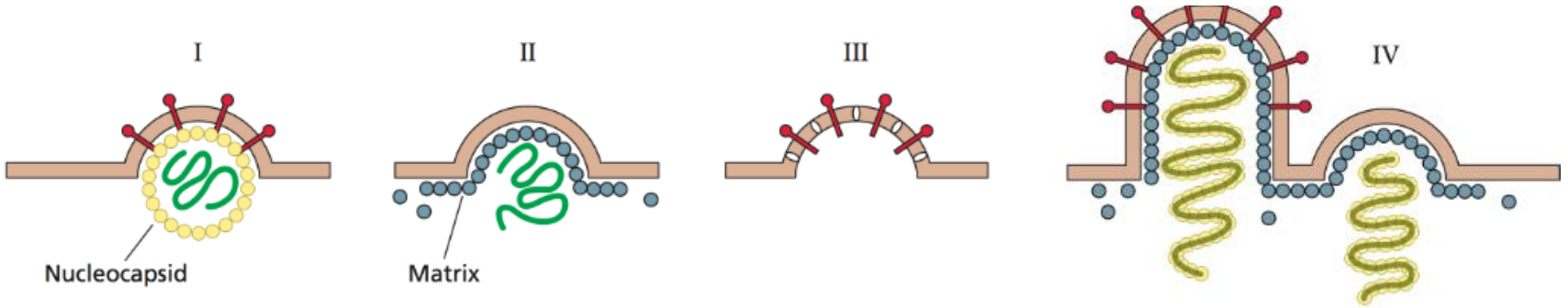
Challenges enveloped vs. non-enveloped viruses

Enveloped viruses: contain envelope derived from the host cell membrane

Non-enveloped virus: plasma membrane as a barrier?

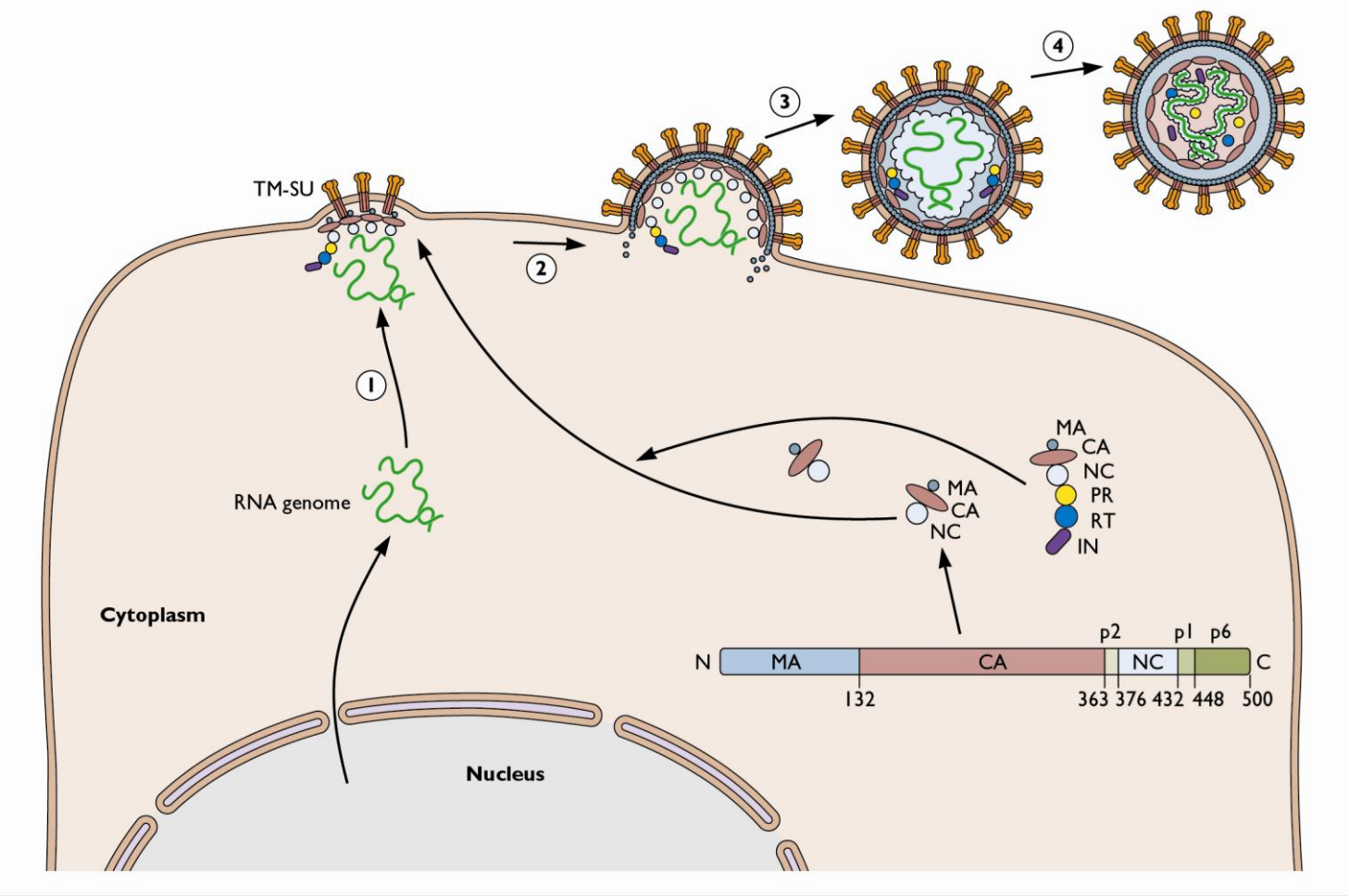
Release of enveloped viruses

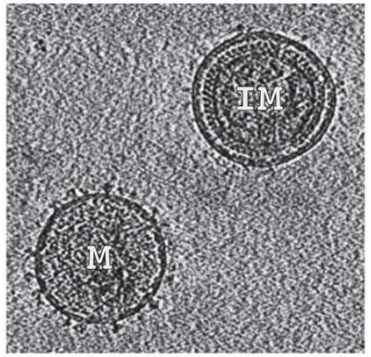
after assembly of internal structures (most enveloped viruses) acquire an envelope



Envelope/ Glycoproteins, Capsid	**Internal matrix Or Capsid proteins**	**Envelope proteins**	**Matrixproteins drive budding, but additional components (glycoproteins, RNP) needed for efficiency or accuracy**
essential for budding	**drive budding**	**drive budding**	
Alphaviruses	**Retroviruses**	**Murine hepatitis virus**	**Orthomyxovirus**

Non-segmented enveloped virus: e.g. retroviruses





Prasad et al., 2022

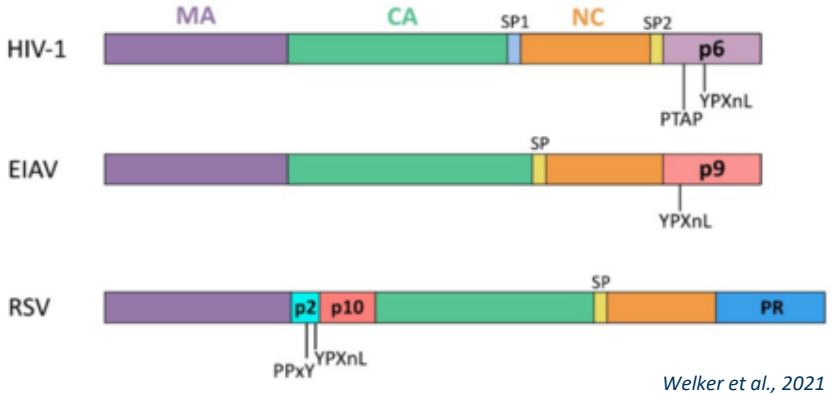
1. **Gag polyprotein** of all retroviruses contains MA, CA and NC proteins

2. **Association of Gag molecules at PM** with one another and with the **RNA genome initiated budding**

3. Incorporation of further Gag molecules, **release of immature non-infectious particle**

4. **Cleavage** of Gag and Gag-Pol by viral protease to produce mature **infectious particles**

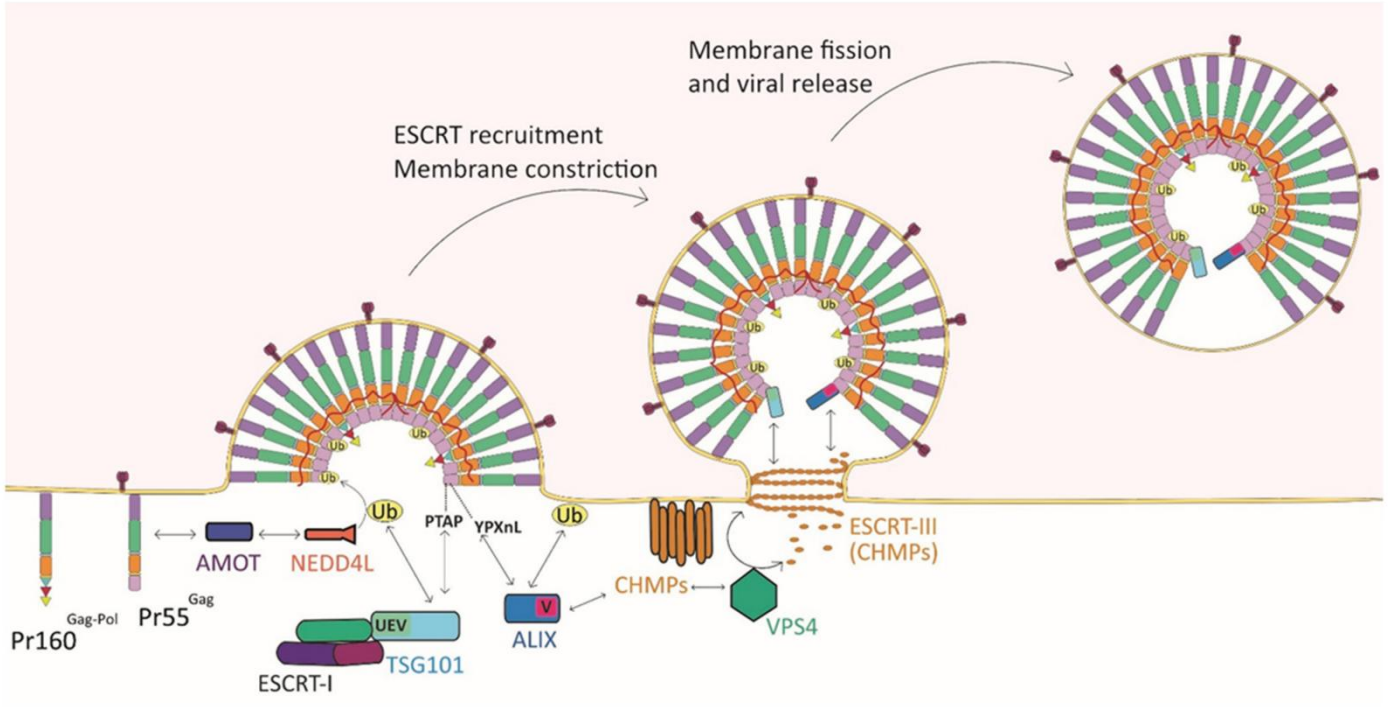
Retroviral Gag proteins contain late domains



Welker et al., 2021

Late domains interact with **vacuolar protein sorting** proteins / **ESCRT machinery** involved in vesicular trafficking

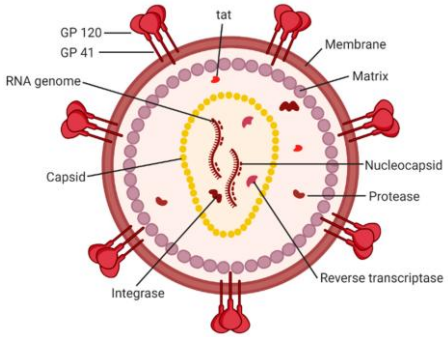
PTAP: TSG101
YxxL: Alix (apoptosis linked gene 2 interacting protein)
PPPY: **Nedd4** ubiquitin ligases



Welker et al., 2021

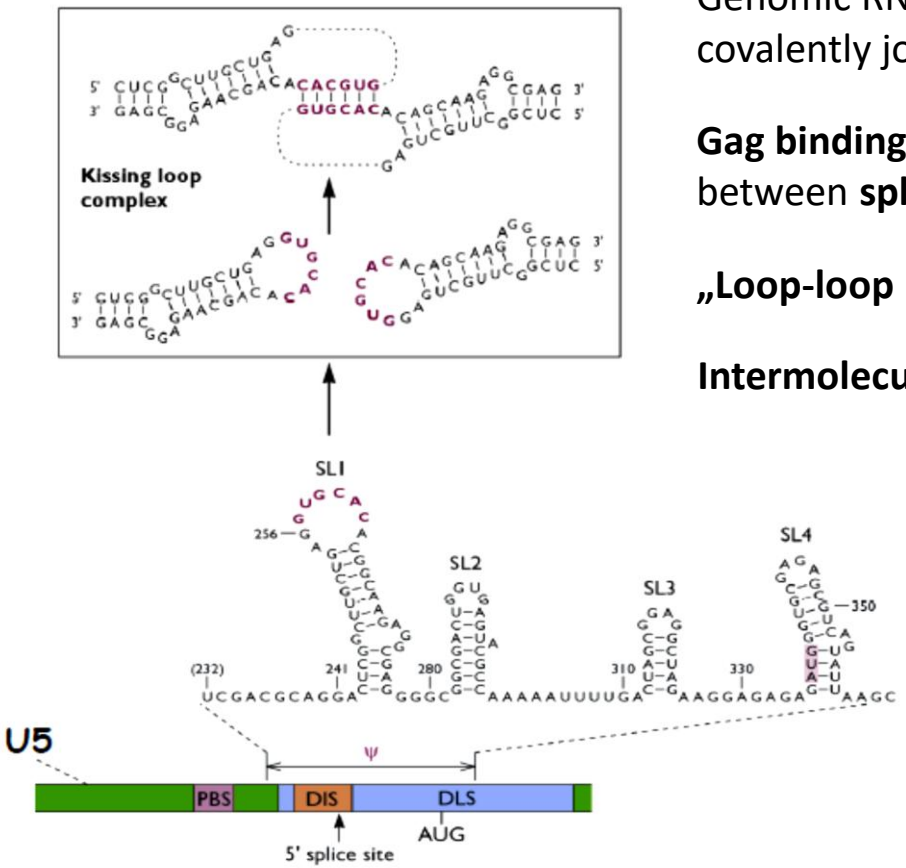
Packaging of viral genome: Retroviruses

Problem: Viral genomes must be distinguished from cellular DNA/RNA where assembly takes place → **packaging signals**



Retrovirus
ssRNA genome (as dimer)

RNA-dimerization



Genomic RNAs are **packaged as dimers**, non-covalently joined through their 5´ends

Gag binding to ψ (packaging signal) → distinguish between **spliced viral RNA** and **genomic RNA**

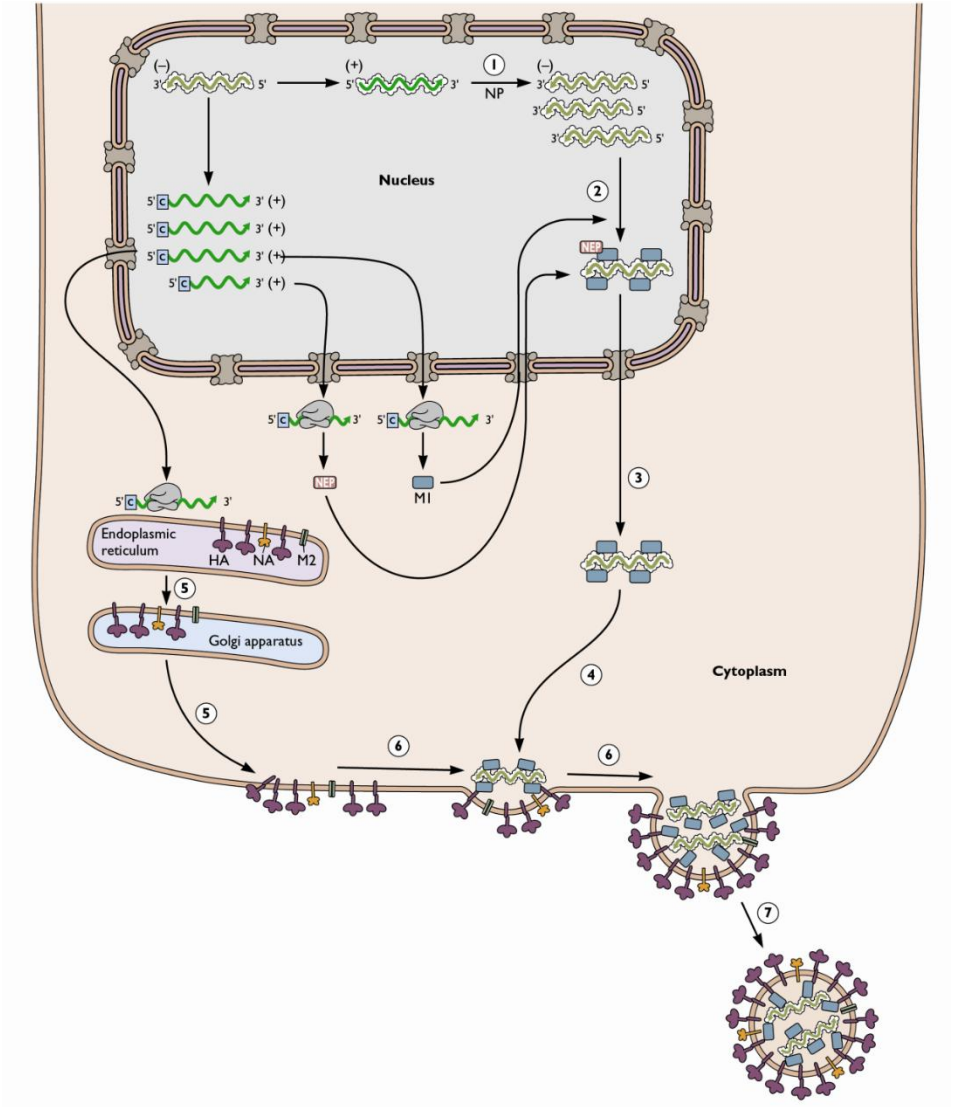
„Loop-loop kissing complex“

Intermolecular bonding due to self complementary sequences

DIS: Dimerization initiation site (palindromic sequence)

DLS: Dimer linkage sequence

Segmented enveloped virus: e.g. influenza virus



1. **Genomic RNA** is synthesized in the **nucleus**

2. **Packaging** by the NP-RNA-binding proteins **(nucleocapsids)**

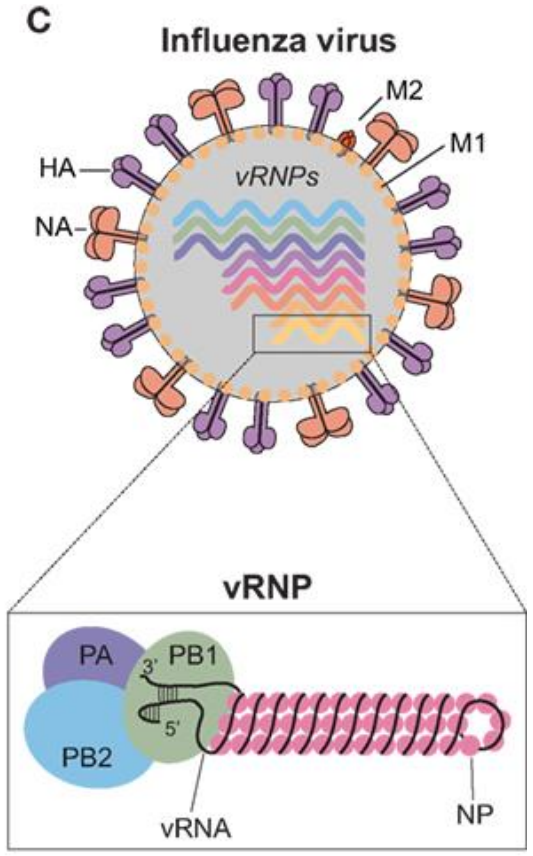
3. Binding to **M1 protein prevents further transcription or replication** and allows binding of NEP (nuclear export protein)

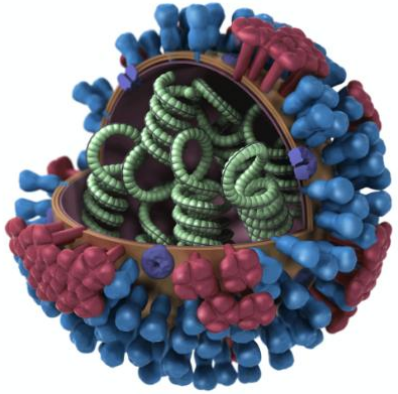
4. **Export of nucleocapsid** to the cytoplasma

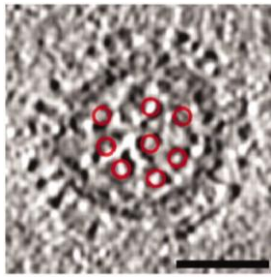
5. M1 also binds to **plasma membrane** which carries **HA, NA and M2** proteins

6. **M1** is the driving force of **budding**

Genome packaging of segmented genomes: e.g. influenza virus







Harris et al., 2006

Influenza virus: **8 different RNA segments**

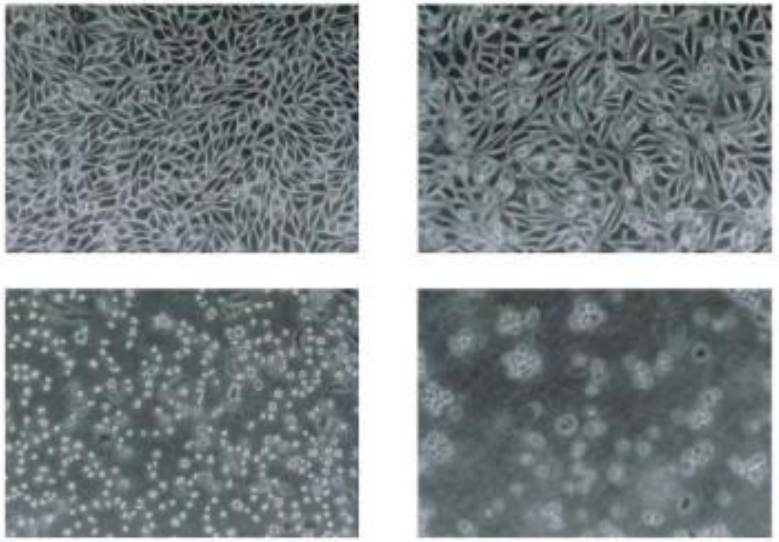
How is the **correct number of RNA segments inserted** into newly synthesized virus particles?

Two different mechanisms: **random** and **selective packaging**

- ***Random* mechanism** would yield 1 infectious particle per 400 assembled - within known particle:pfu ratio ($8!/8^8$)
- Evidence for **specific packaging sequence** on each RNA segment

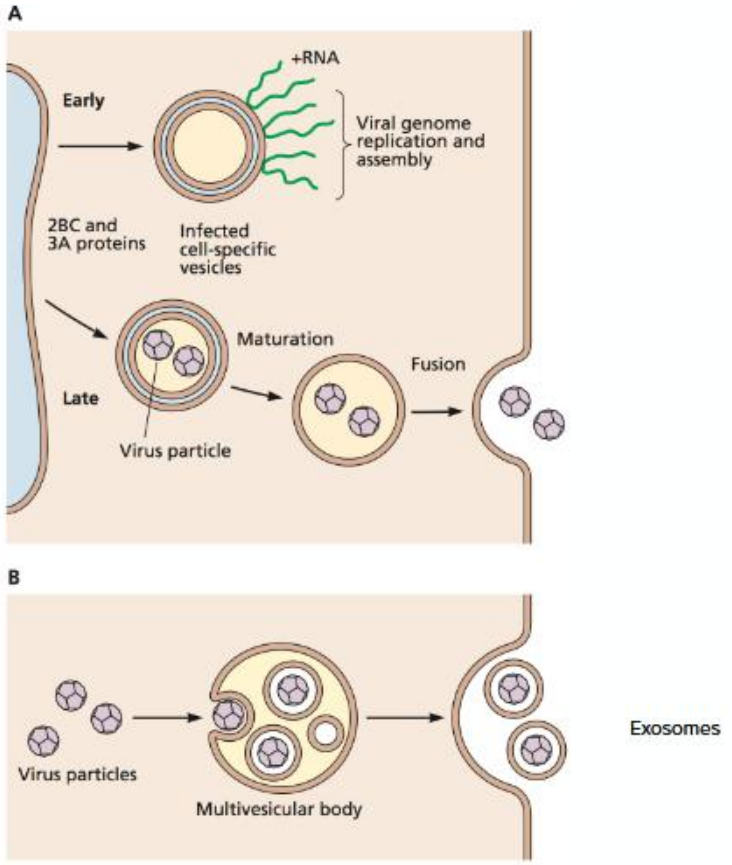
http://www.virology.ws/2009/06/26/packaging-of-the-segmented-influenza-rna-genome/

Release of non-enveloped viruses

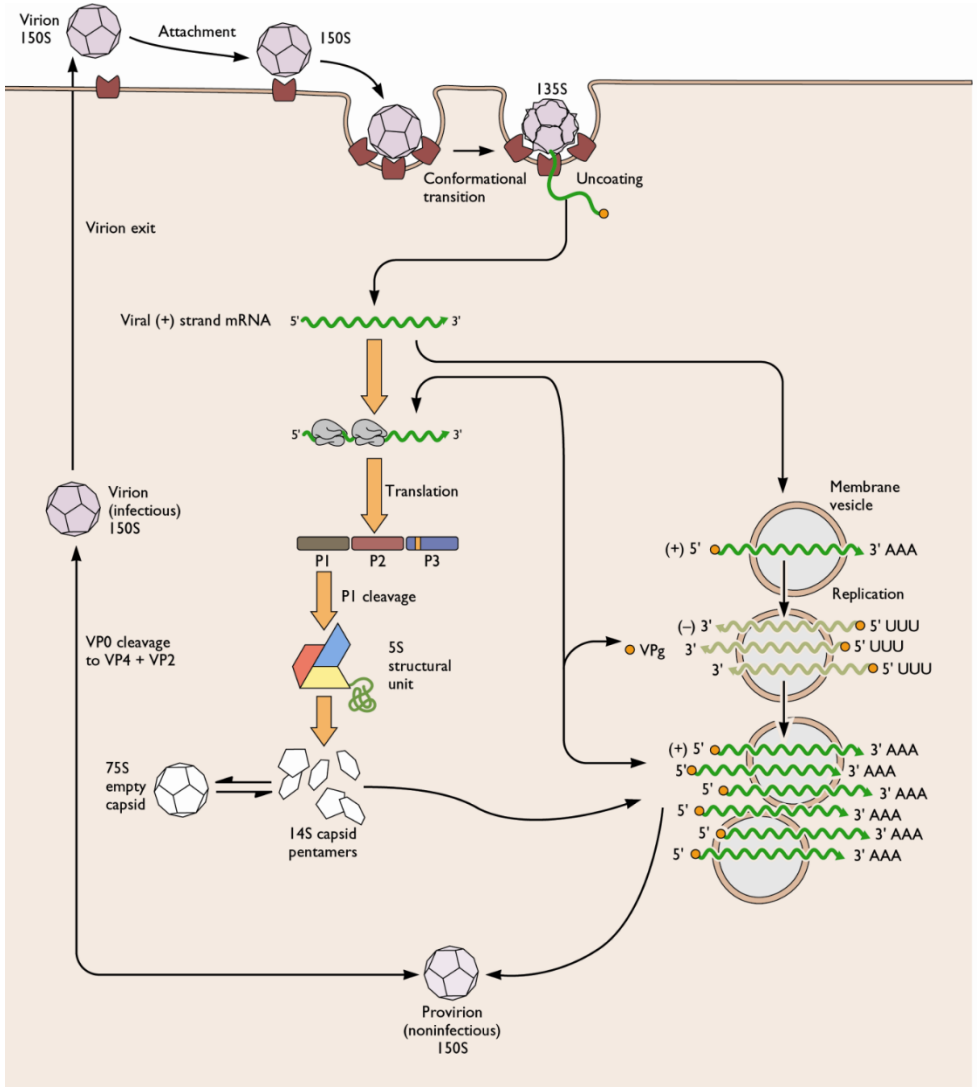


- **Cell lysis**: apoptosis, necroptosis
- Viral proteins that induce **rupture of cell membranes**
  - Viroporins form **pores in cell membranes** (polyomavirus)
- **Loss of membrane integrity** with inhibition of protein synthesis

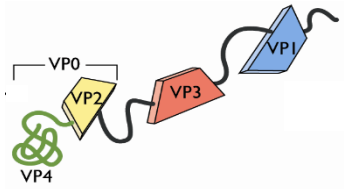
Non-lytic release of non-enveloped viruses



Poliovirus assembly and release



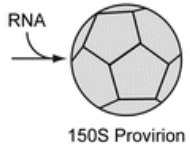
Poliovirus assembles from polyprotein precursor

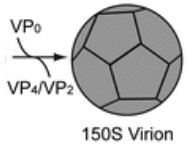


1. Formation of **immature structure 5S subunit (VP0, VP1, VP3)**

2. **75S empty capsids storage** forms of 14S pentamers

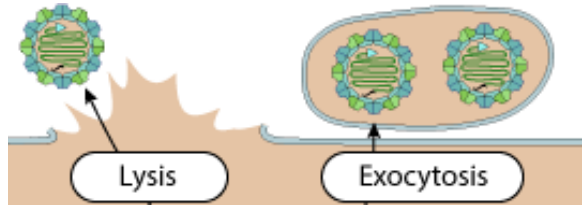
3. Formation of **capsid shell from 14S pentamers** is coordinated with genome encapsidation and requires replication **(150S non-infectious)**



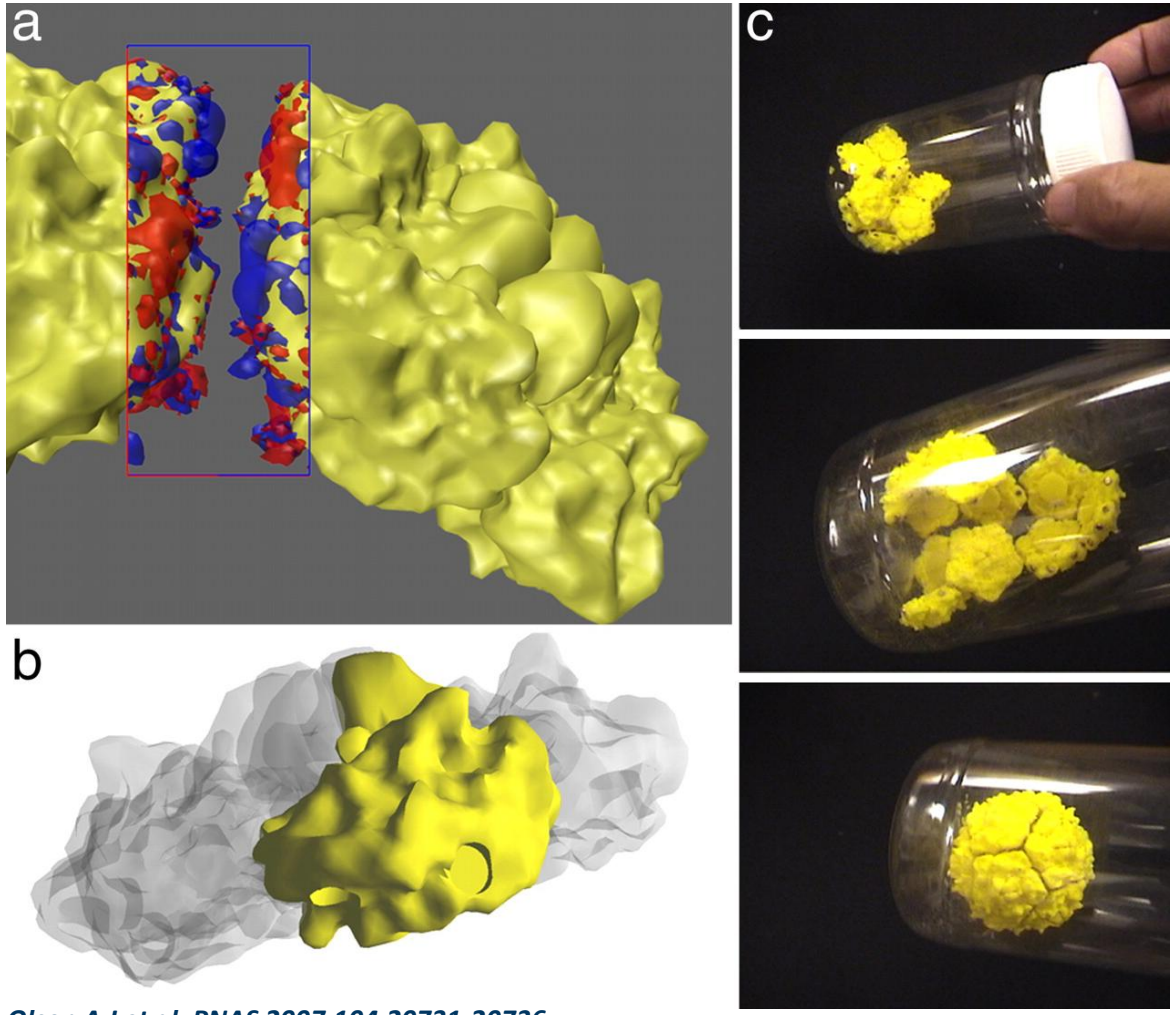


4. **VP0 cleavage** to VP4 and VP2 **infectious virion released**

5. **Cell lysis** (host cell death) triggered by poliovirus accumulation or **vesicular release** (packets of virus in vesicles)



From molecular structure to self-assembling plastic model



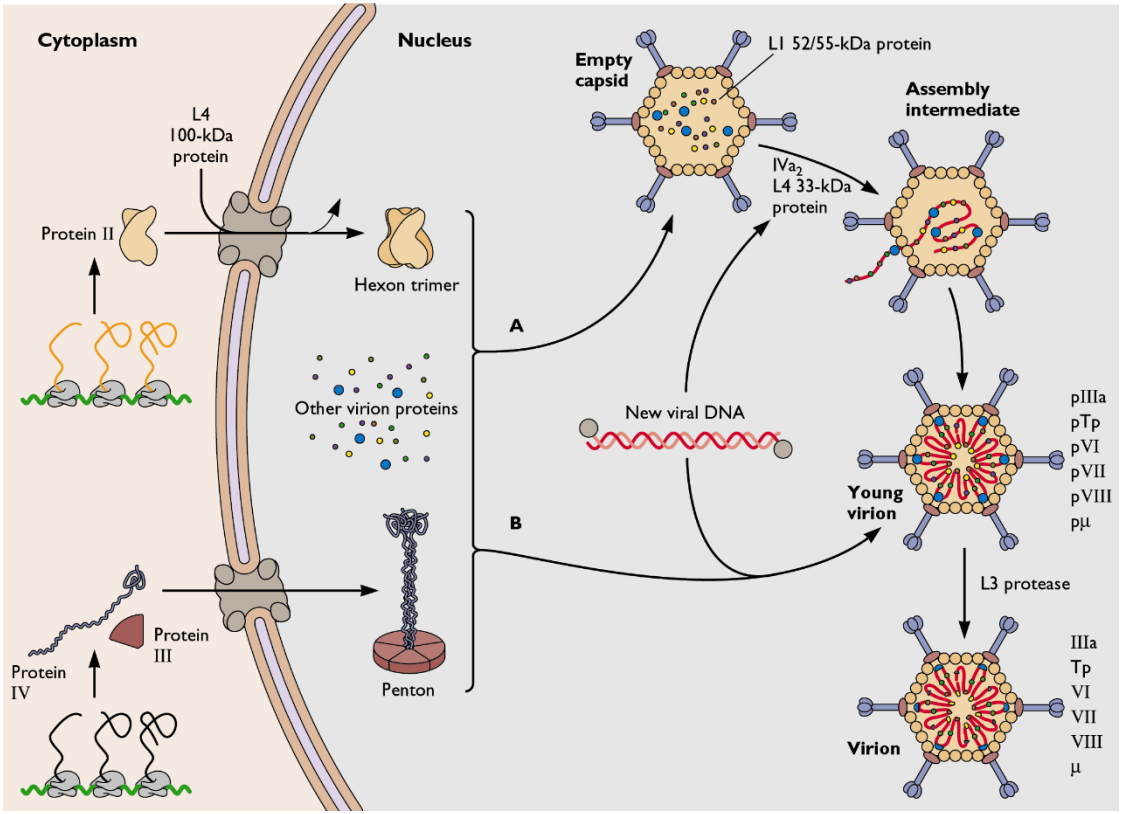
Olson A J et al. PNAS 2007;104:20731-20736
For movie: http://www.pnas.org/content/suppl/2007/12/05/0709489104.DC1

Poliovirus pentameric **assembly intermediates**

Geometry used to make autofabricated pentameric tile

Sequence showing **self-assembly by shaking** of the 12 tiles into a complete capsid

Adenovirus assembly and release



Adenovirus assembles from individuel protein molecules

1. Synthesis and assembly of **hexons** and **pentons, transport into nucleus**

2. **L4 protein required** for formation of hexons

A 3. Structural units and proteins assemble into **empty capsids**

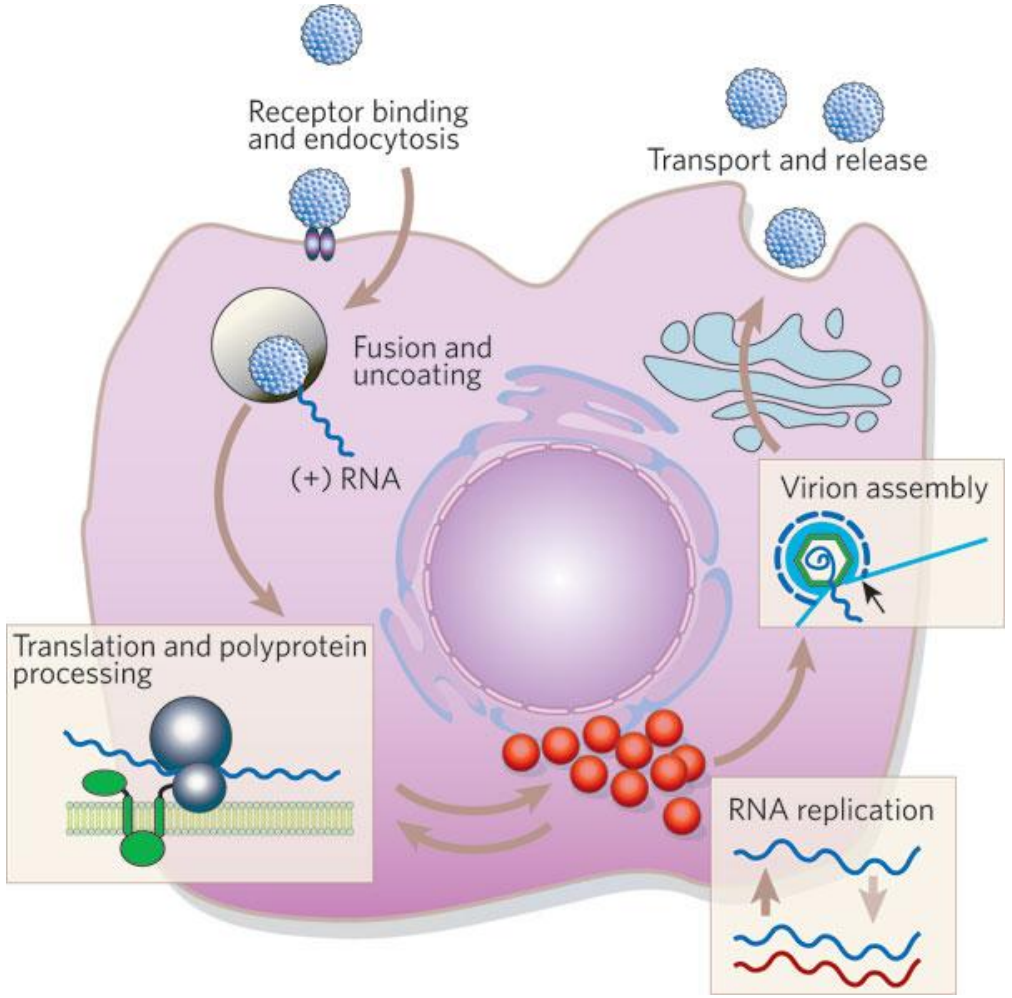
4. IVa2 binds to **packaging signal of genome**: assembly intermediate

5. **Mature particles** are produced upon **cleavage of the precursor proteins**

B 3. Based on the **failure of any capsid-like structures** to assemble (mutations)

4. Capsid assembly and encapsidation of the genome are **concerted reactions**

Overview of the Hepatitis C Virus (HCV) life cycle as an example



You tube: http://hcvlifecycle.univ-lyon1.fr

Summary

- **Assembly**: multiple reactions **coordinated, irreversible**
- **Assembly**: attractive as **antiviral target**
- **Diversity** in size, composition and structural sophistication
- **All viruses** must complete a common set of ***de novo*** **assembly reactions** to ensure reproductive success
- Viral structures suited for **protection of the nucleic acid genome;** built in a way that allows their **ready dissassembly during entry**
- **Very stable** association among virion components **during assembly and transmission, but reversal** of these interactions when appropriate signals are encountered **upon infection of a new host cell**