RUHR-UNIVERSITÄT BOCHUM

RUB

Assembly, Egress and Maturation of Viruses

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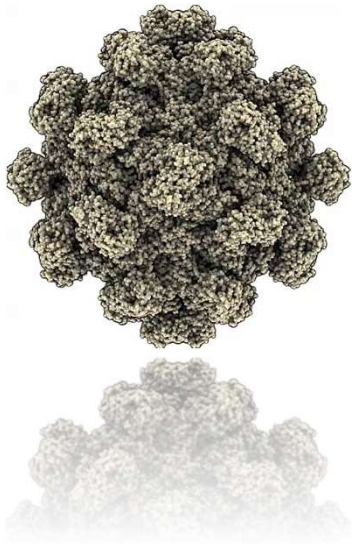
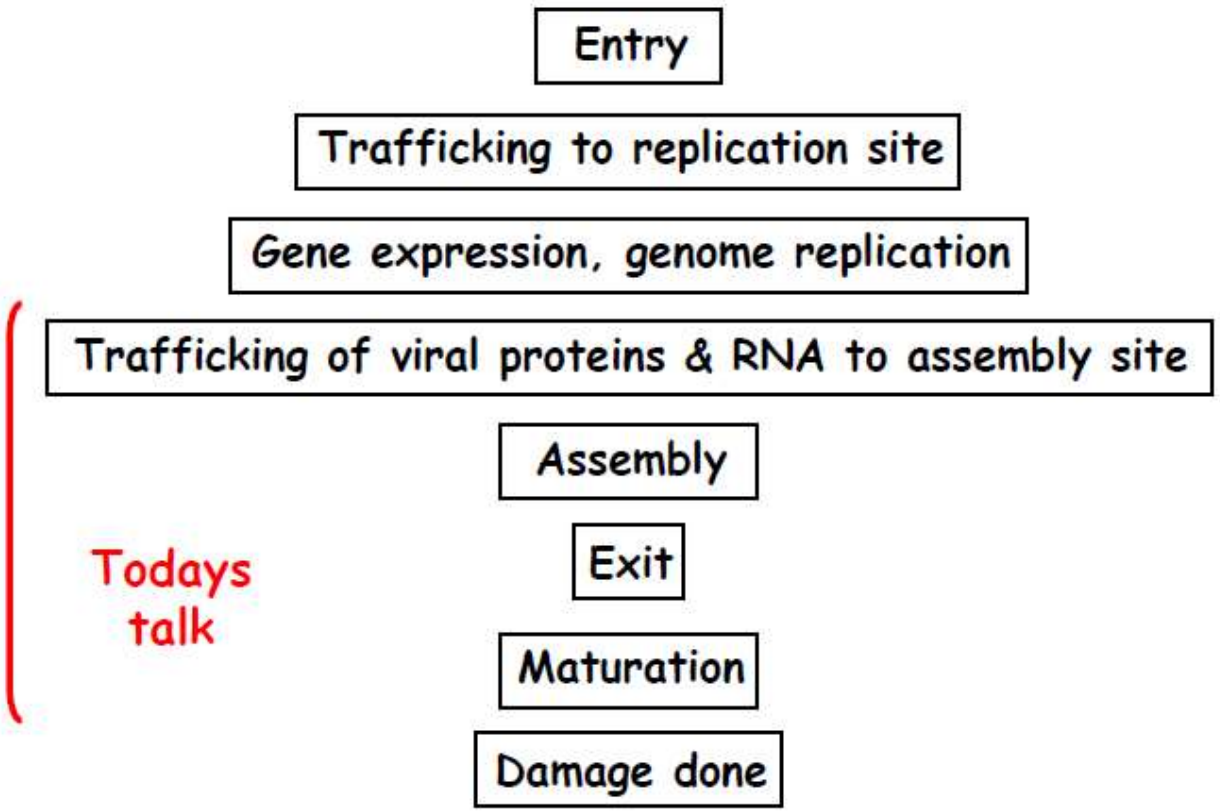
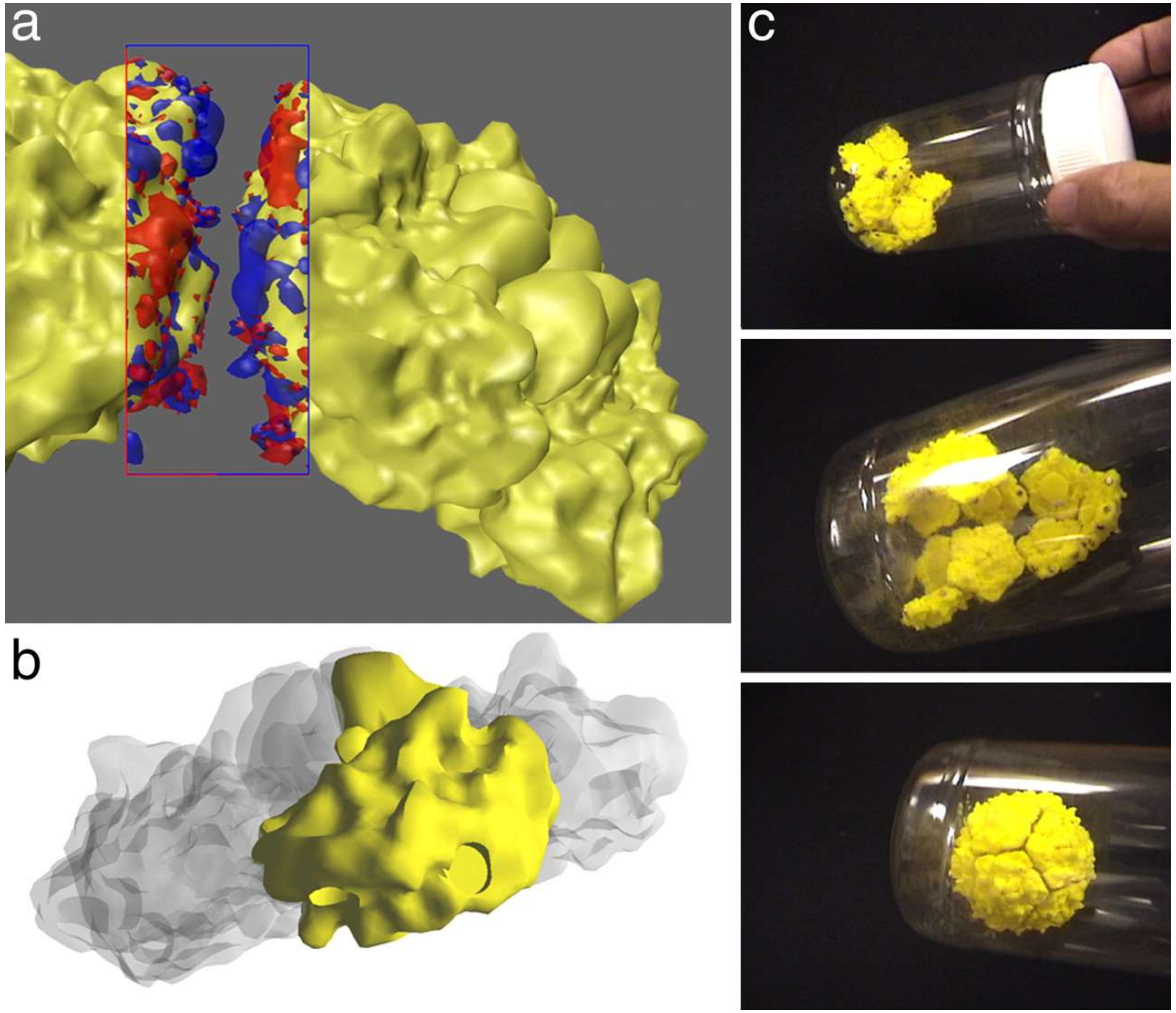
Molecular & Medical Virology



The “ins and outs” of a virus



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

Requirements for directed trafficking of viral proteins and nucleic acids

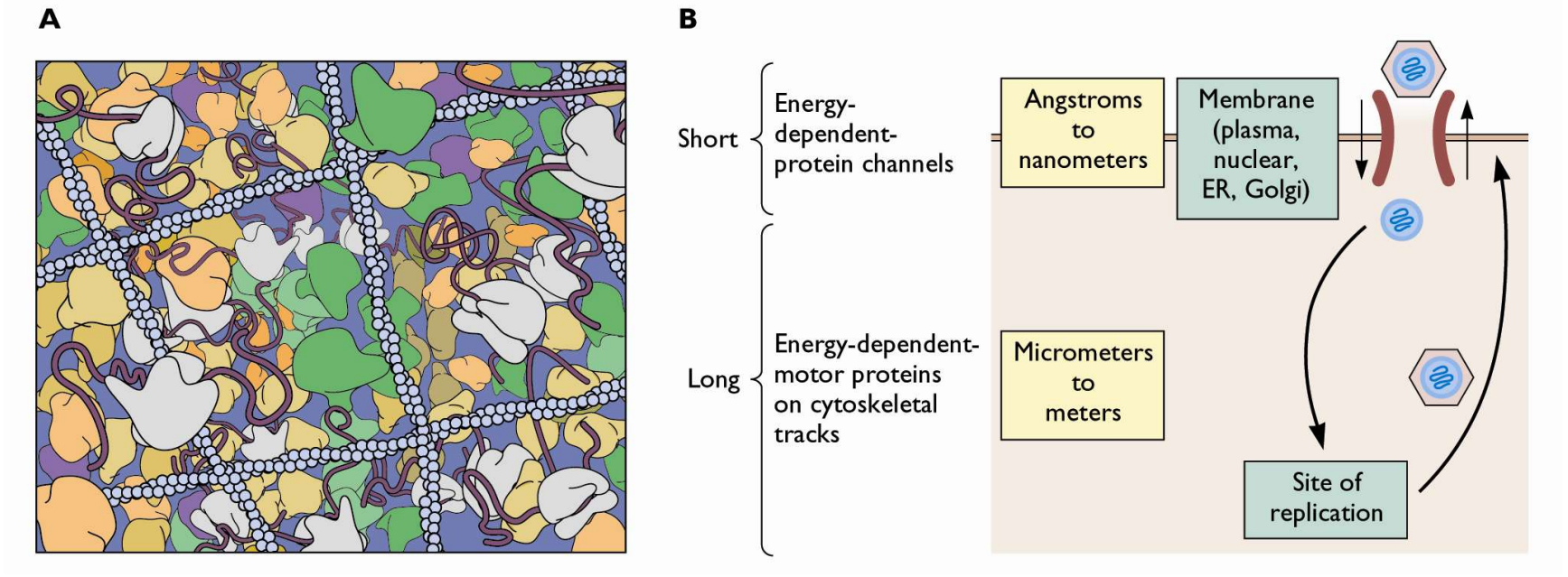
Table 12.1 Intracellular trafficking requirements for virus assembly

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

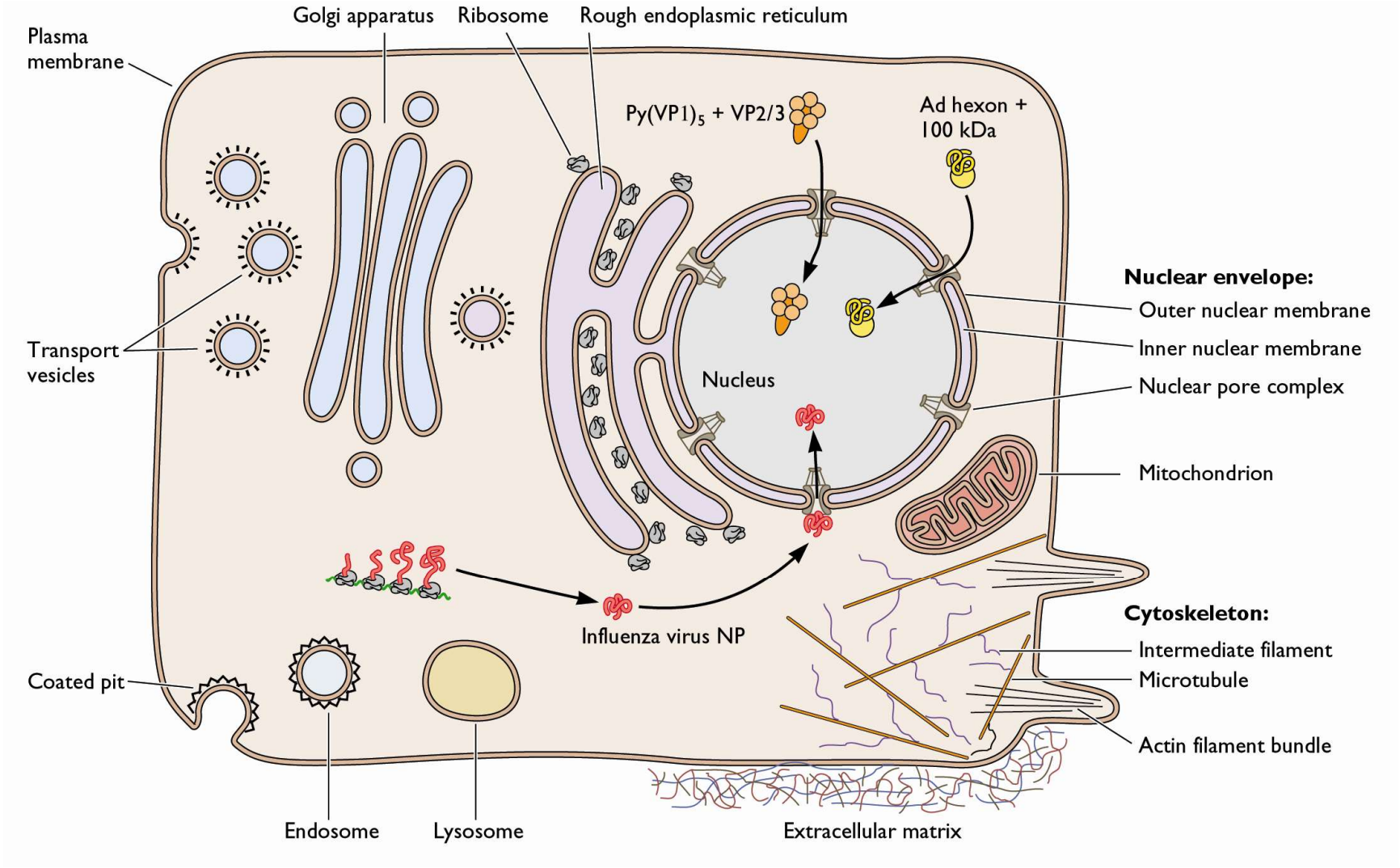
Directional movement

Short range: Crossing a membrane, release from a capsid: Channels

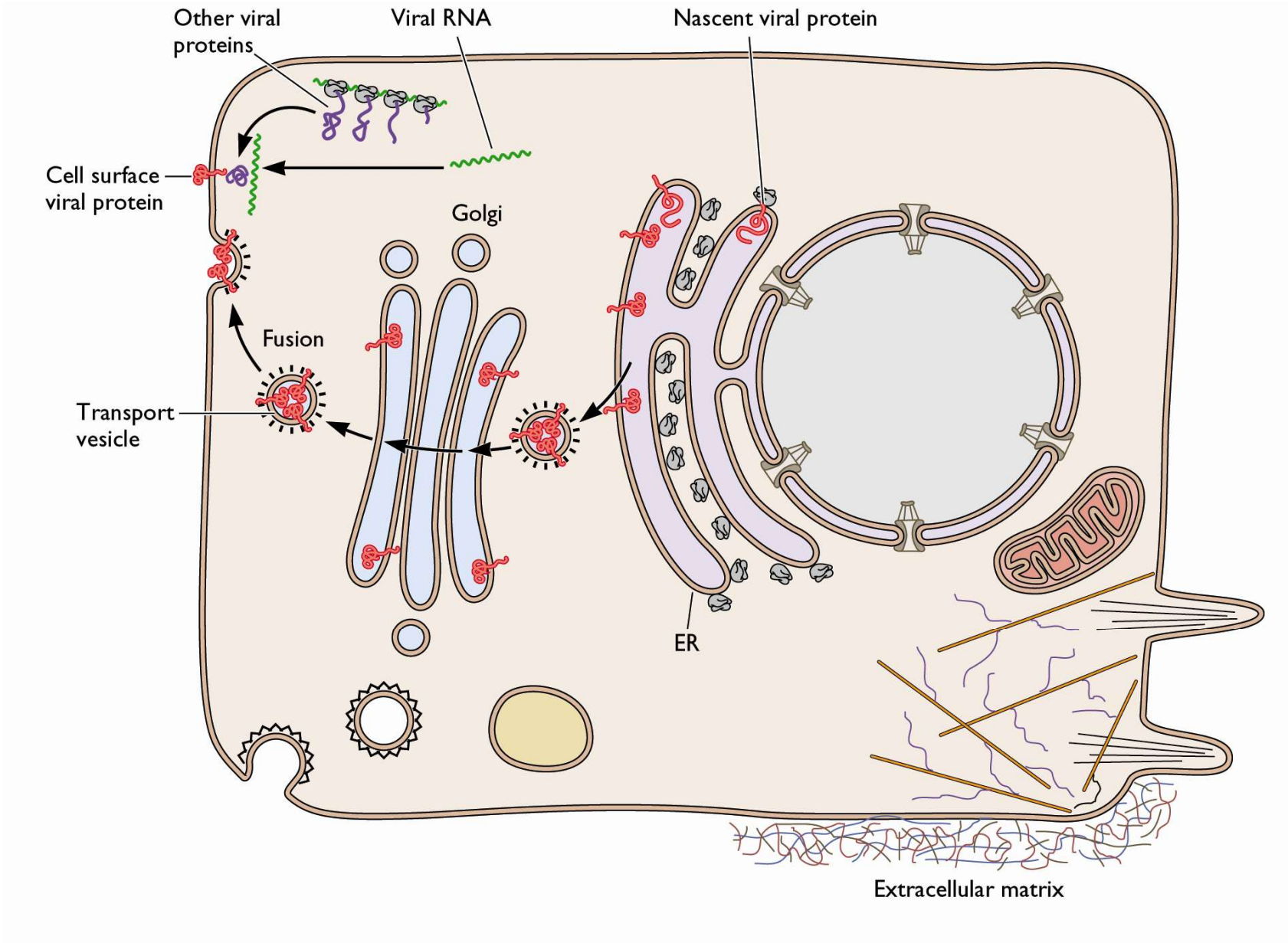
Long range: Movement only cytoskeletal tracks:
Myosin motor/actin
Dynein/kinesin motor/microtubules



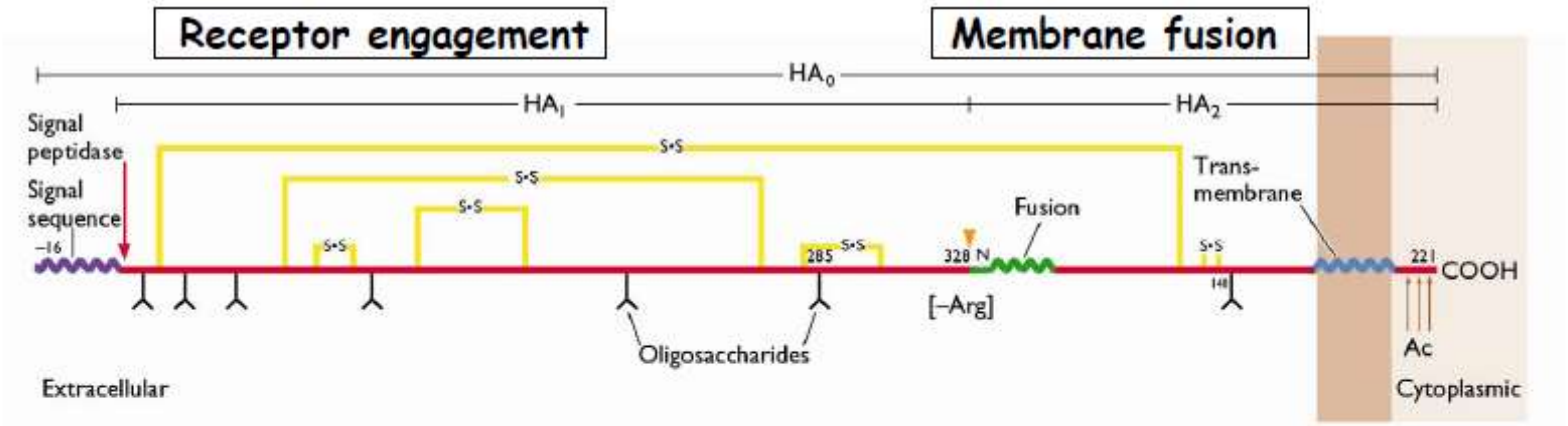
Localization of viral proteins to the nucleus



Localization of viral proteins to the plasma membrane



Assembly at the plasma membrane: transport of viral glycoproteins



Signal peptide	Glycans	Disulfide Bonds	Cleavage site	Membrane Anchor
ER import	Folding Immune protection	Folding	Activation	Complete membrane fusion

Biogenesis of viral glycoproteins in the secretory pathway

ER import

Glycosylation, folding, disulfide bonds, oligomerization

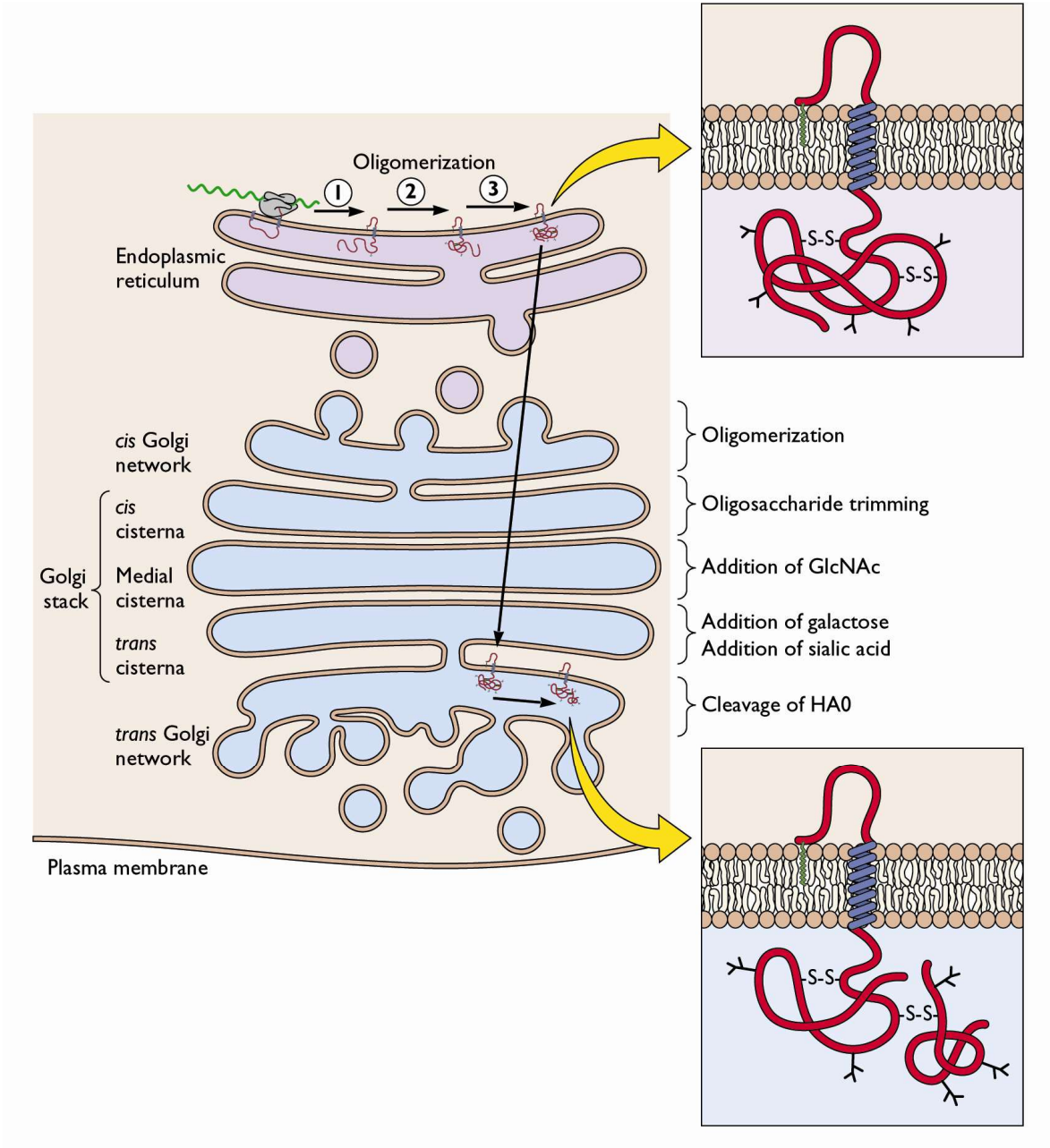
Transport into Golgi apparatus

Trimming of glycans

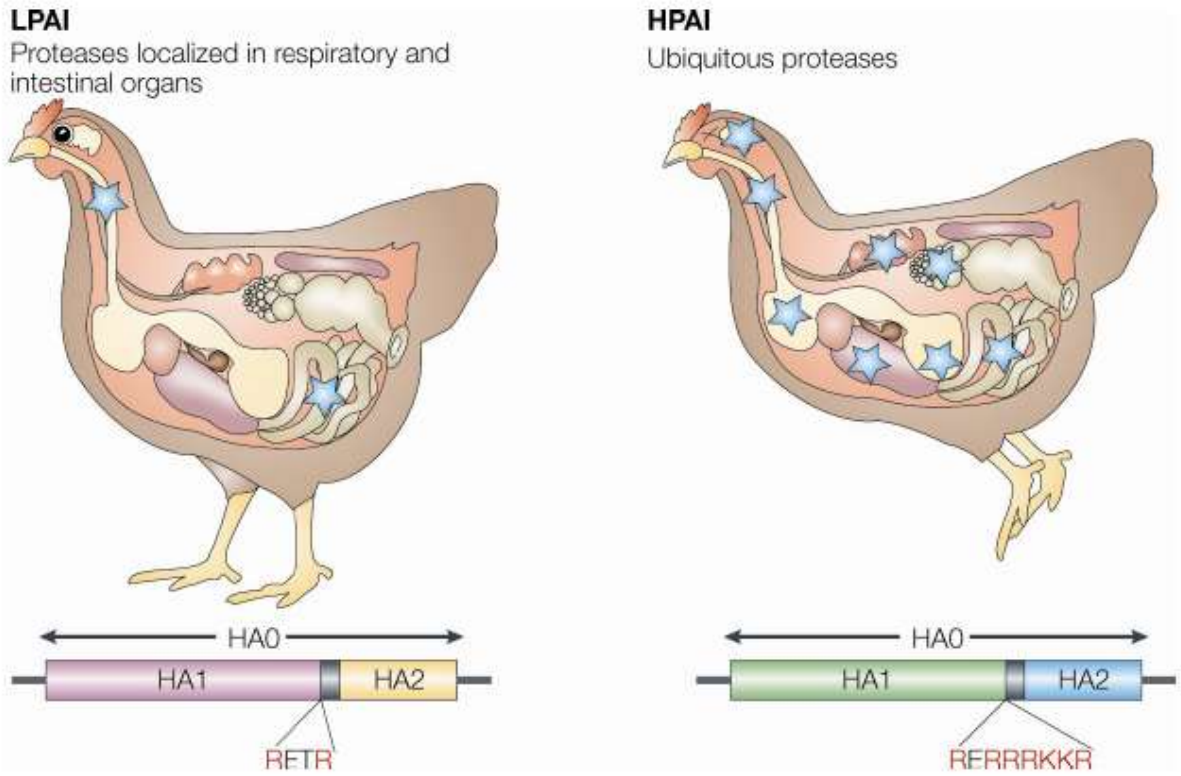
Cleavage

Transport to the plasma membrane

Maturation of influenza virus HA protein along the secretory pathway



Avian viruses: HA cleavage determines pathogenicity



Requires trypsin in culture

Trypsin independent

Targeting of a nascent protein to the ER membrane

- mRNA encoding a protein with signal sequence (ER)

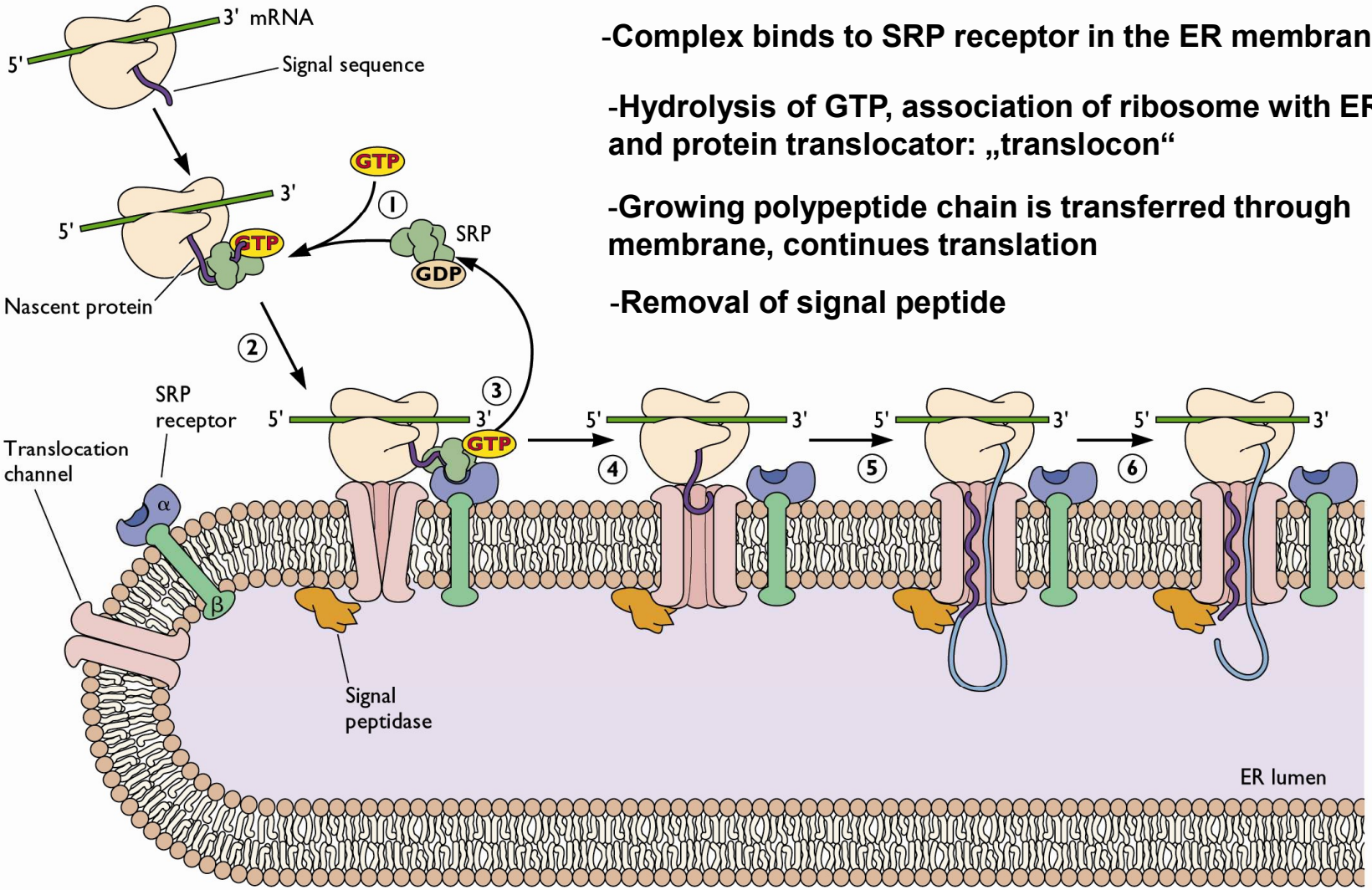
- SRP binds to sp and ribosome (GTP)

-Complex binds to SRP receptor in the ER membrane

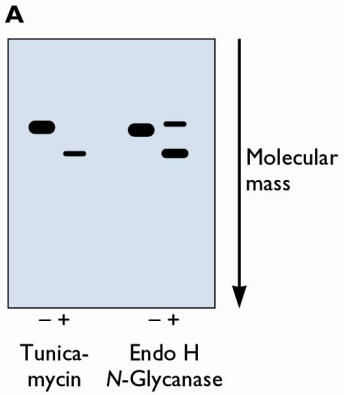
-Hydrolysis of GTP, association of ribosome with ER and protein translocator: „translocon“

-Growing polypeptide chain is transferred through membrane, continues translation

-Removal of signal peptide



Detection and structure of N-linked oligosaccharides

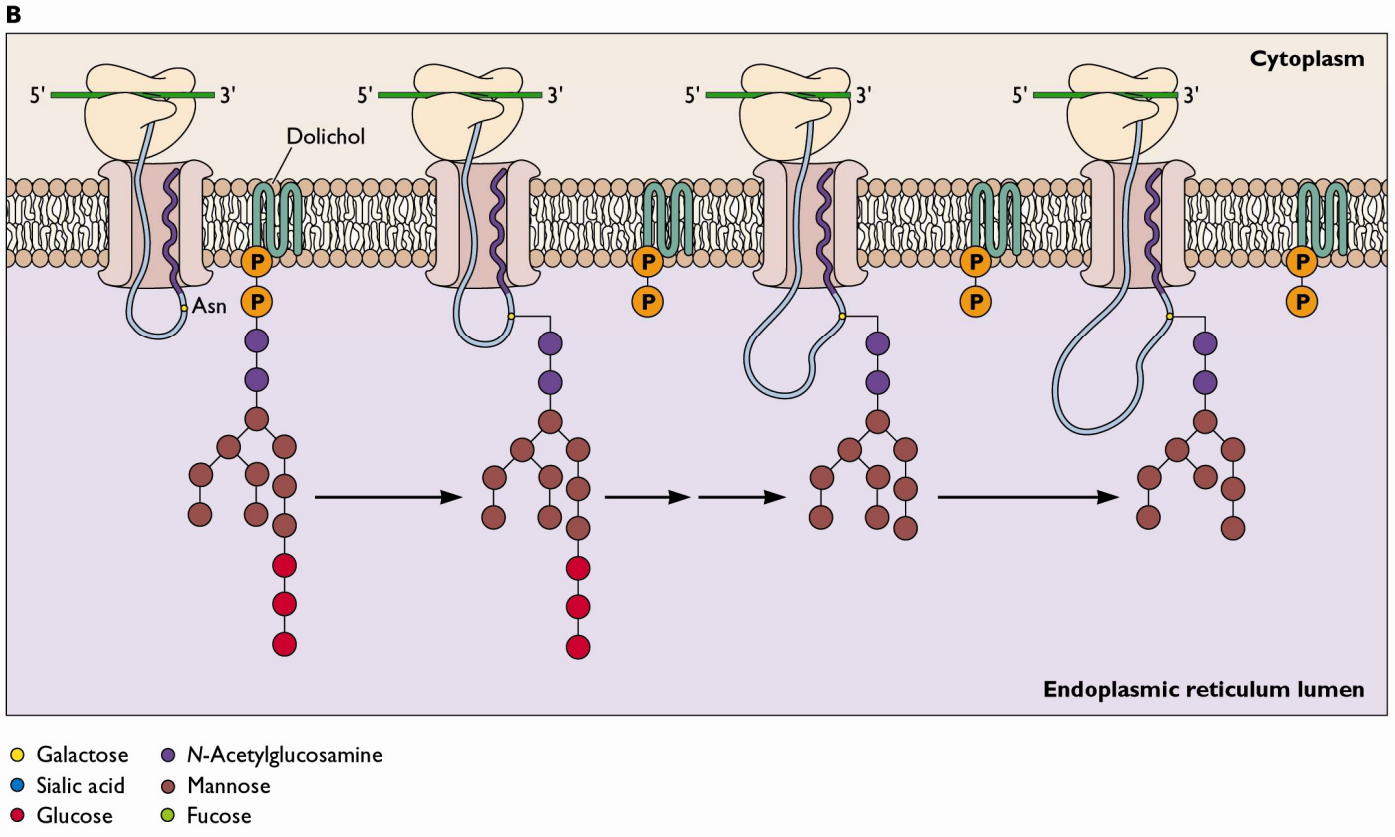


- detection of N-linked oligosaccharides by use of tunicamycin or glycosidases

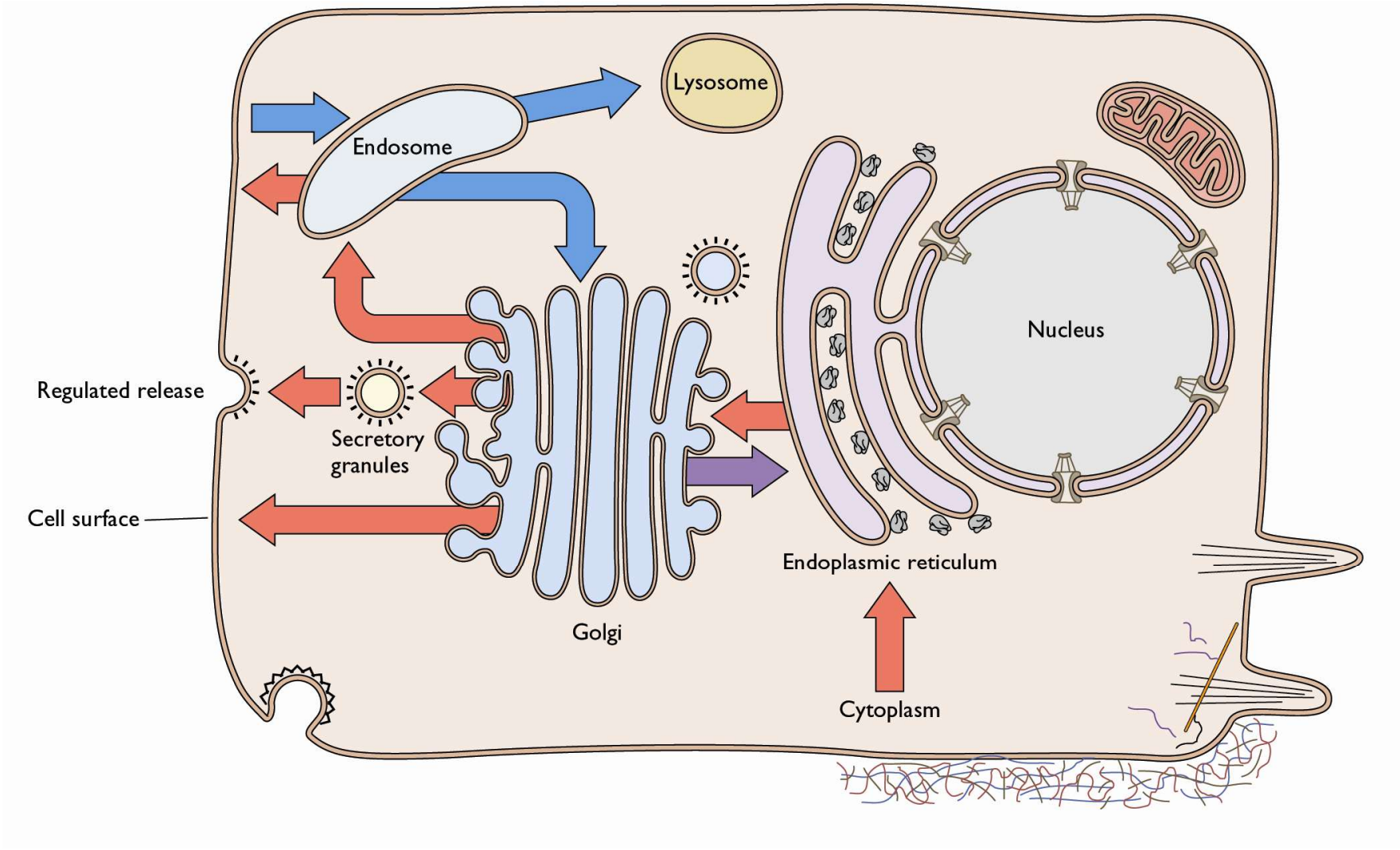
- mannose-rich oligosaccharide added via N-glycosidic bond to asparagine residue shown

-Sugar precursor is transferred to N-linked glycosylation sites as protein translocates into the ER

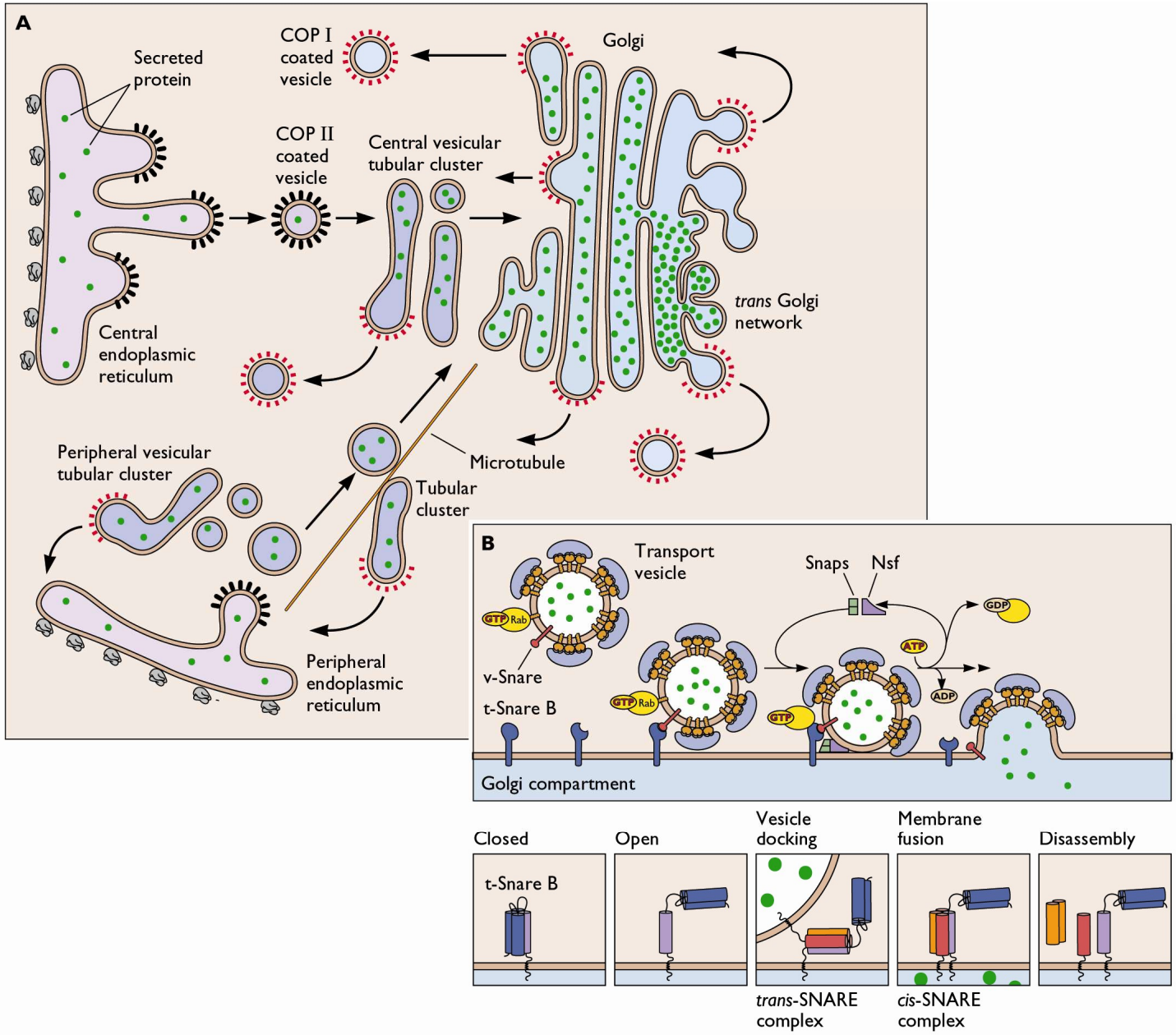
-Three glucose residues and one mannose trimmed and additional sugars are added when protein travels through golgi



Compartments in the secretory pathway



Protein transport from the ER to the Golgi



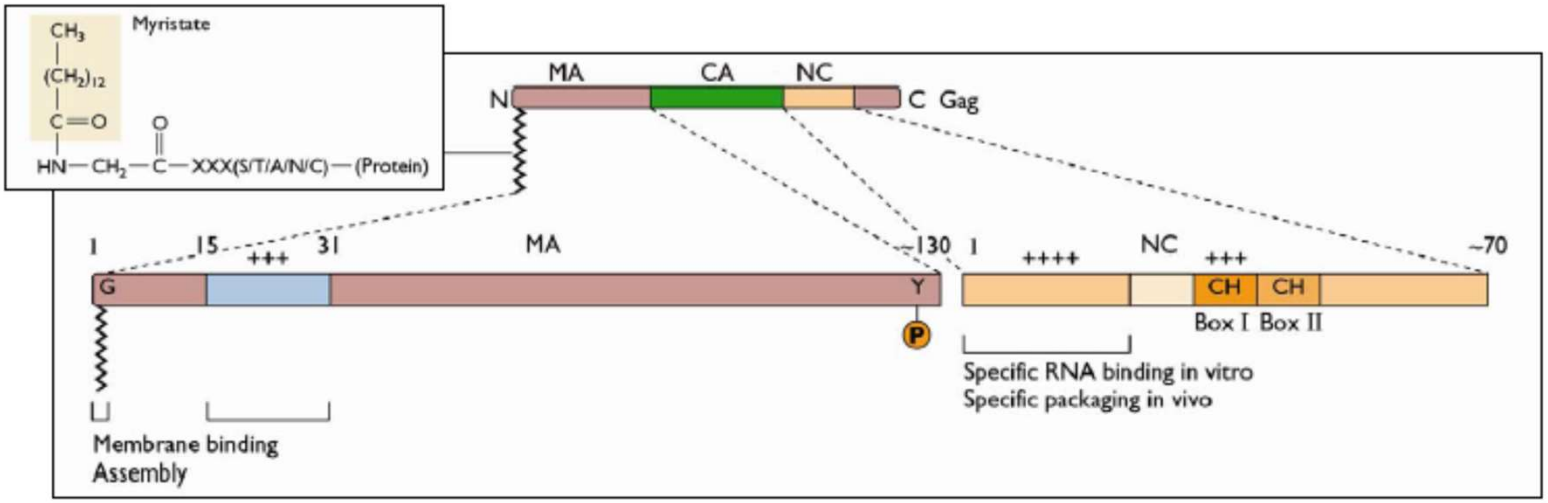
Other means of directed transport of viral proteins

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 viral budding

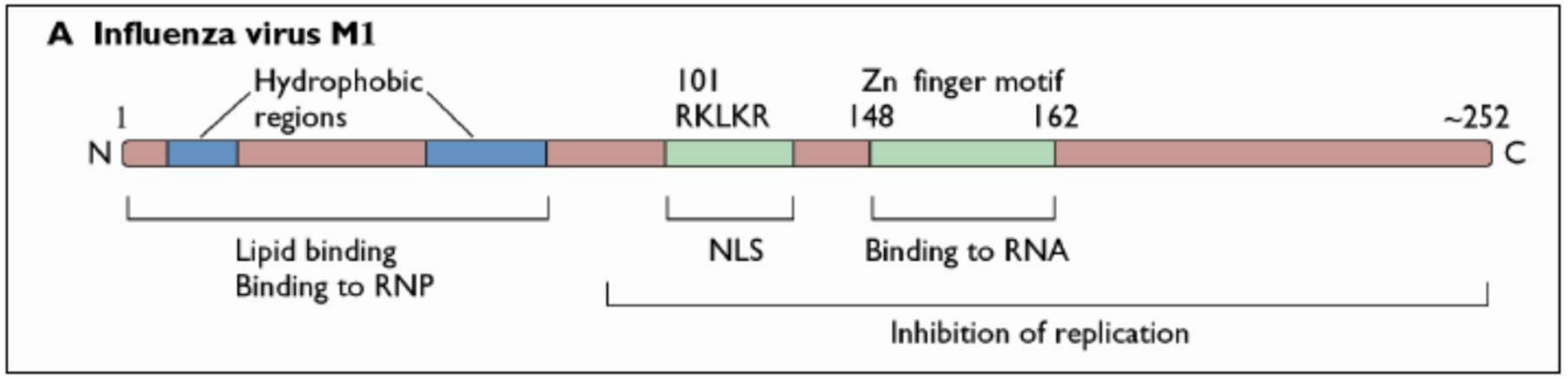
Myristoylation of VP4 is essential for poliovirus assembly



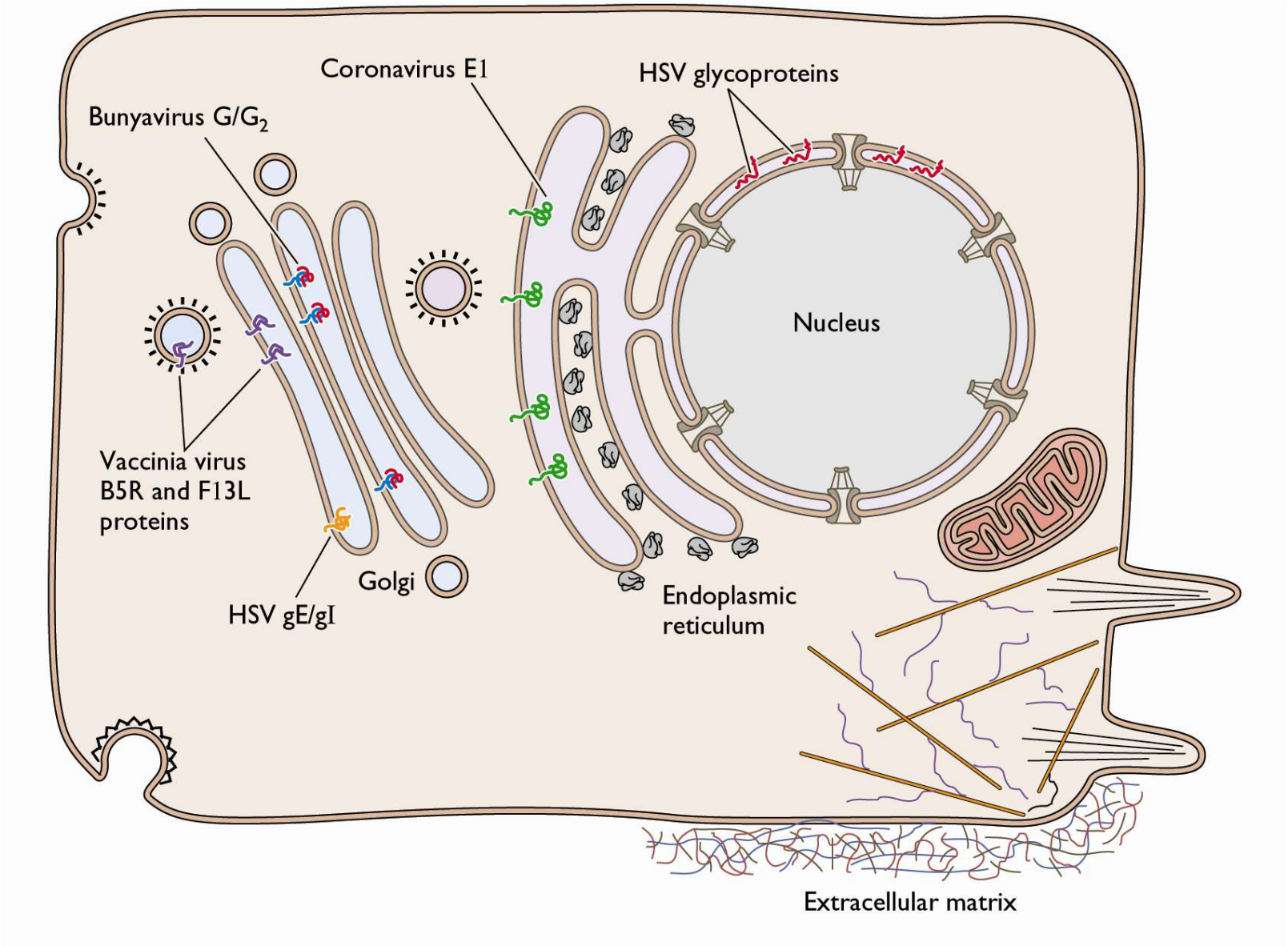
Other means of directed transport of viral proteins

Protein-Signals (SP-independent)

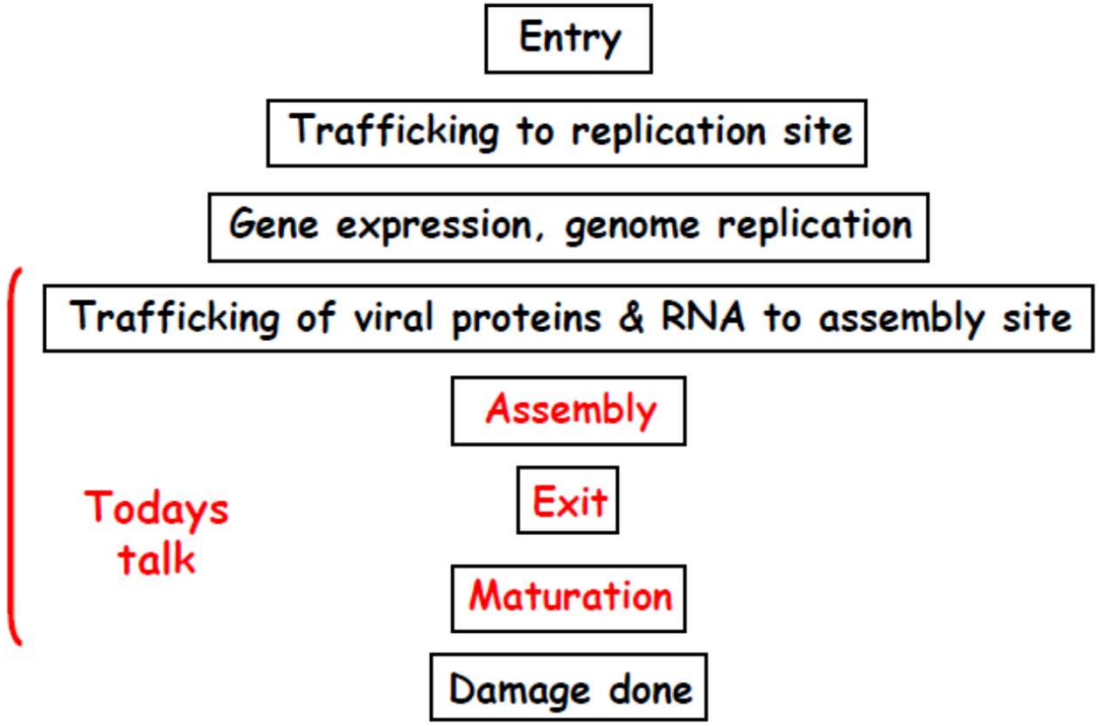
- Matrix proteins of – strand RNA viruses: Essential for genome packaging
- No lipid modification, membrane association intrinsic property of the protein
- Membrane binding maybe enhanced by interaction with viral membrane proteins



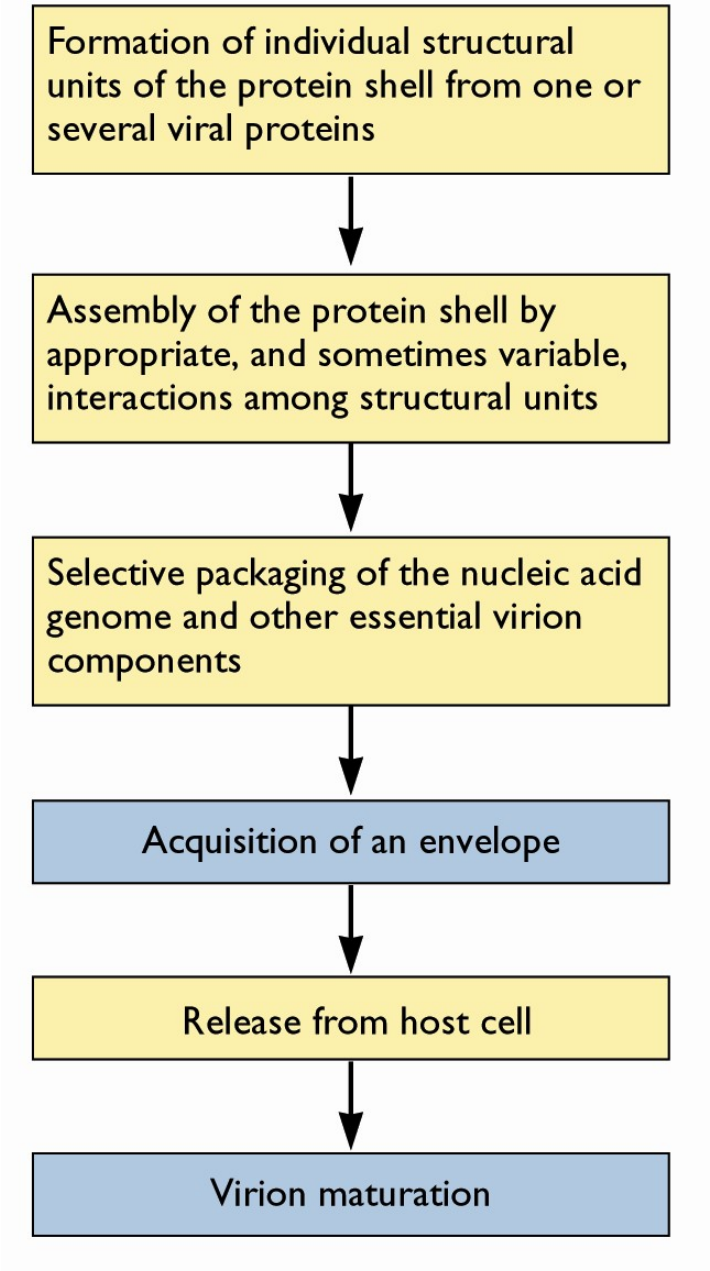
Site of budding depends on localization of envelope proteins



The “ins and outs” of a virus



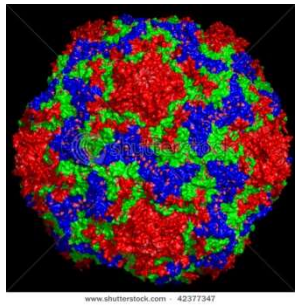
Hypothetical pathway of virion assembly and release



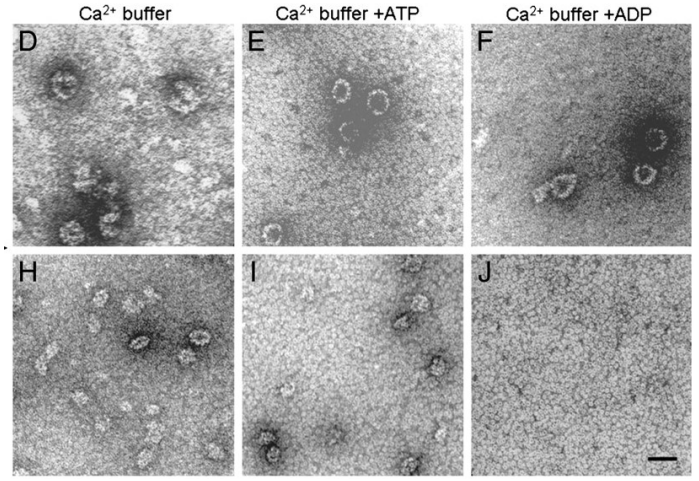
Yellow: common to all viruses

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 (for example *in vitro* translation)

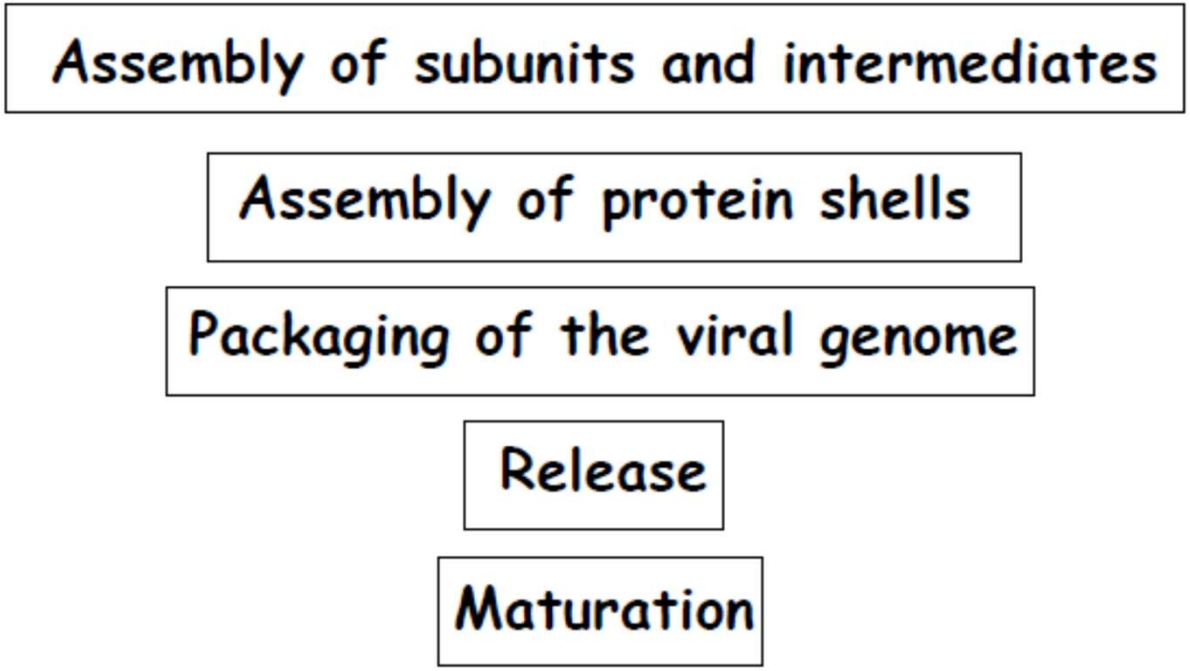


Virus assembly

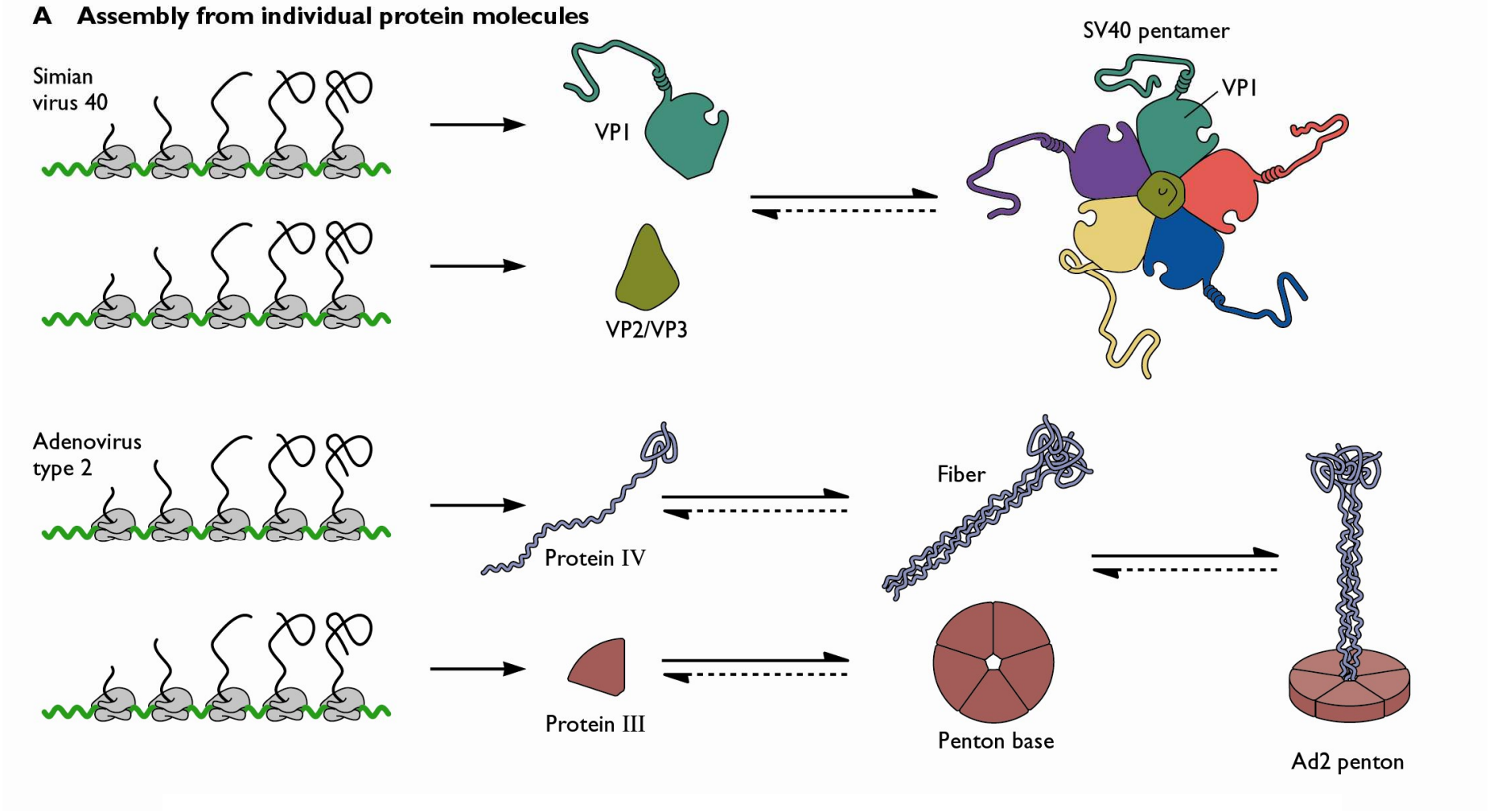
Challenges:

1. Coordination of subunit expression, assembly into structural units and transport to the site of capsid assembly
2. Coordination of capsid assembly and nucleic acid incorporation
3. Capsids: High stability versus efficient disassembly
4. Transport of capsids to membranes harboring envelope proteins
5. Release from the infected cell (budding, lysis) or direct transfer to new host cells

Virus assembly



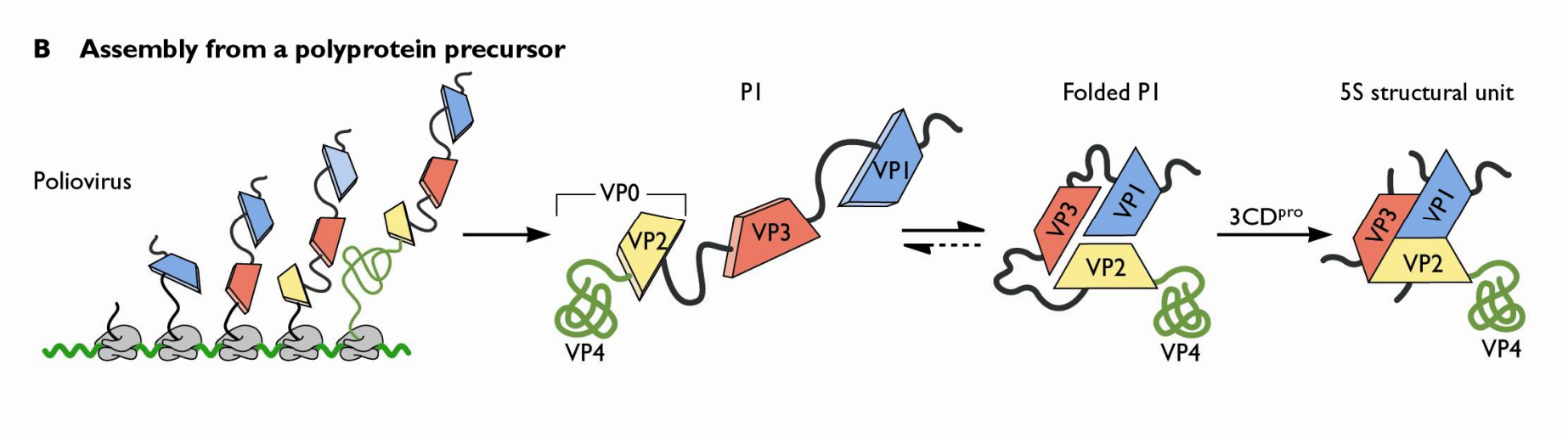
Assembly from individual proteins



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

Assembly from polyprotein: picornaviruses



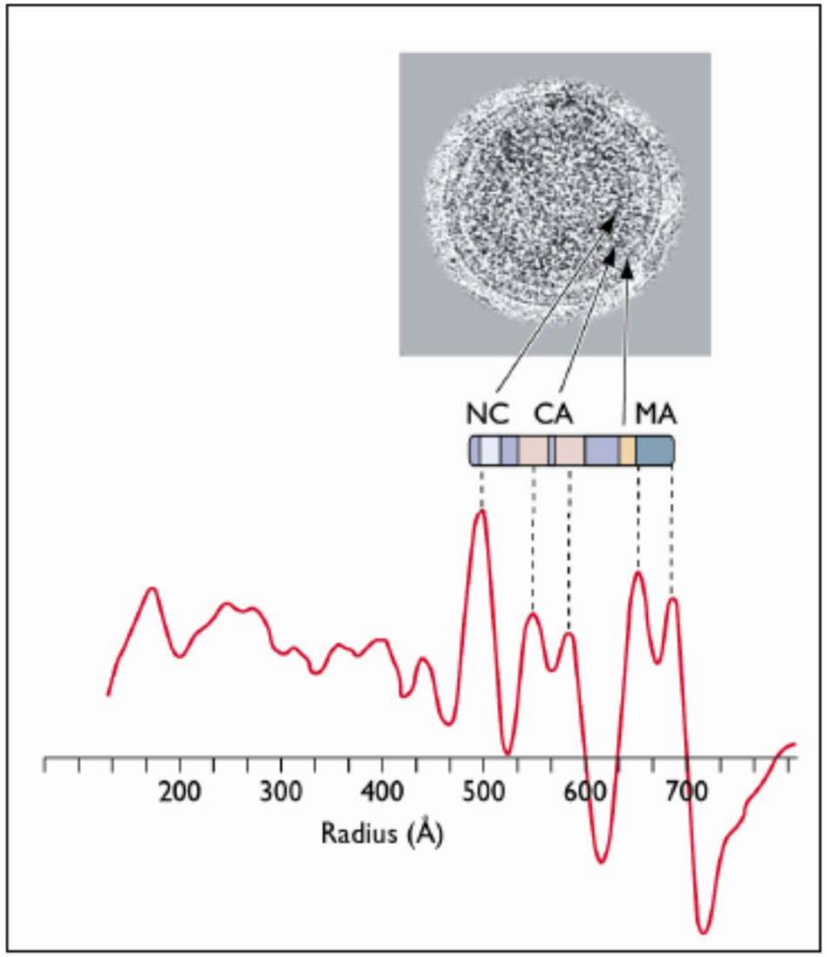
-Four proteins form heteromeric structural unit

-PI immature structural unit: VP0, VP3 and VP1

-Protease allows formation of 5S structural subunit

-VP4 remains covalently linked to VP2 in VP0 until assembly is completed

Assembly from polyproteins: retroviruses



Retroviral Gag proteins:

Polyproteins, cleaved by viral protease in budding virions: **Maturation.**

Maturation is blocked by **protease inhibitors**

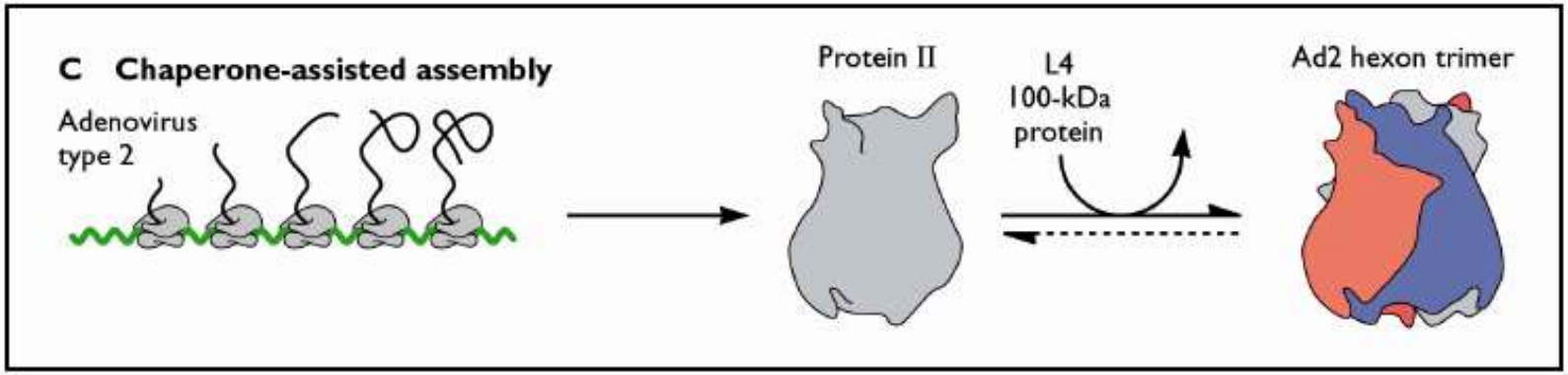
Cellular and viral chaperones

Cellular chaperons required for folding of viral structural proteins:

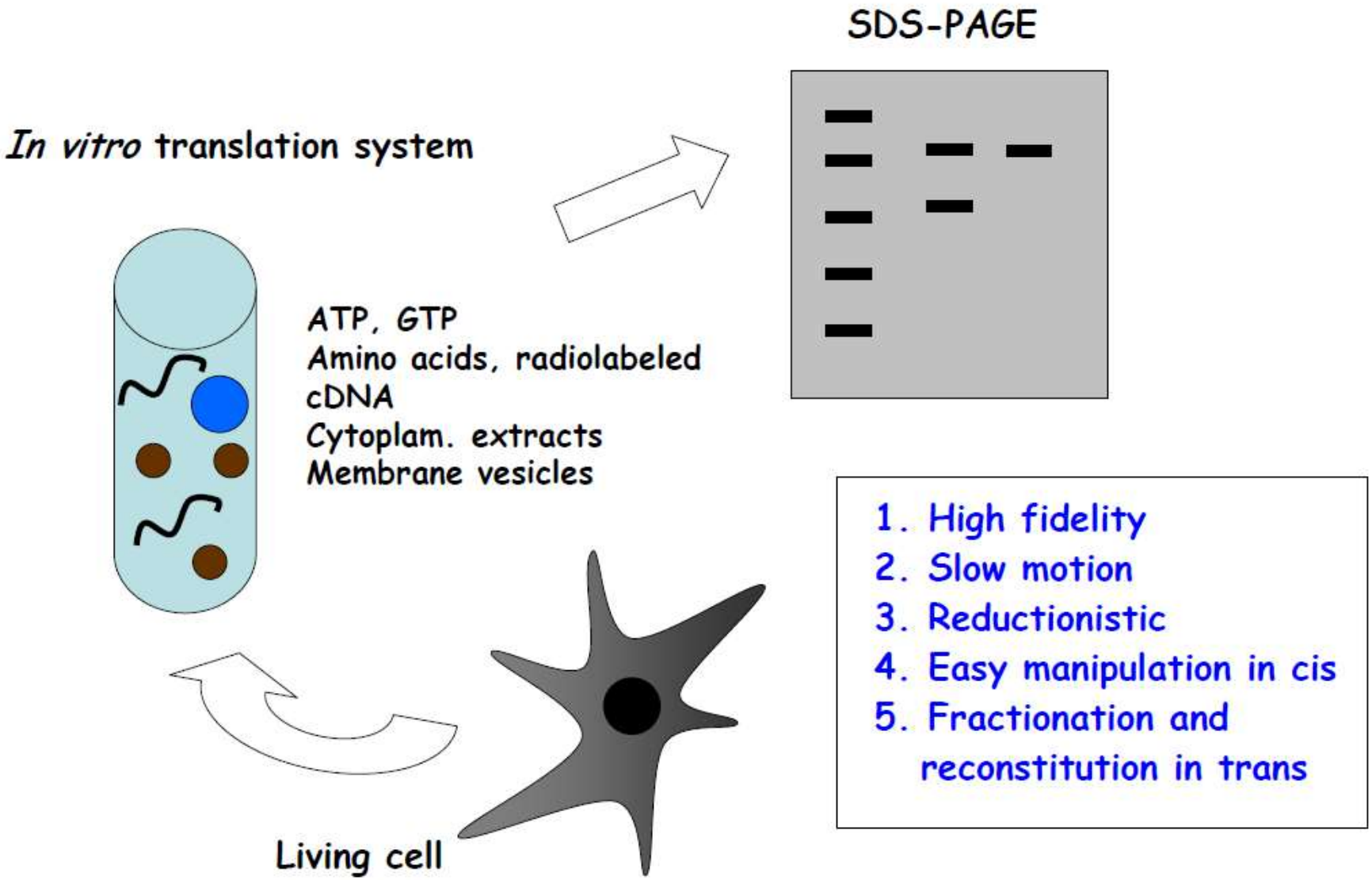
Hsp70 proteins: HIV Gag, Adeno protein IV, HBV L protein
Hsp68 proteins: HIV Gag
Chaperonin TriC: Mason-Pfizer monkey virus Gag

Viral chaperons

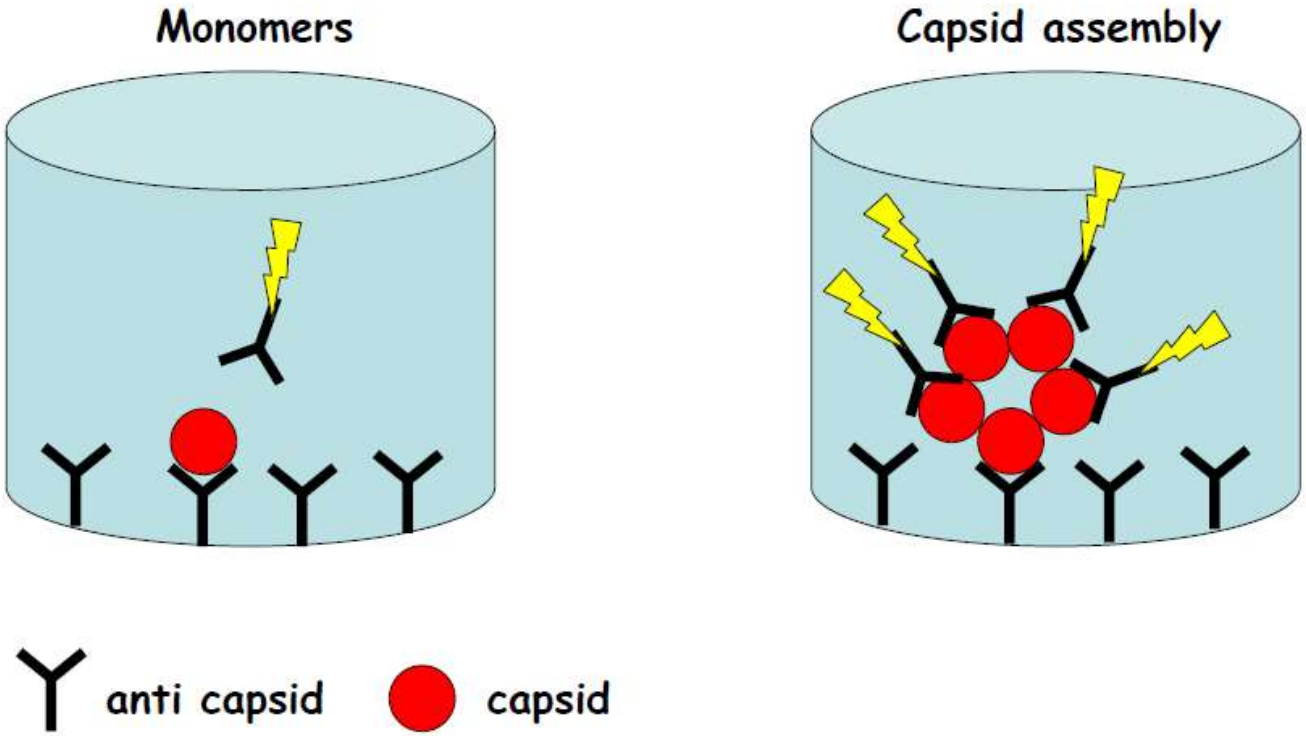
Adeno 2, L4 100kDa protein: Hexon protein
HSV 1, VP22a: VP5



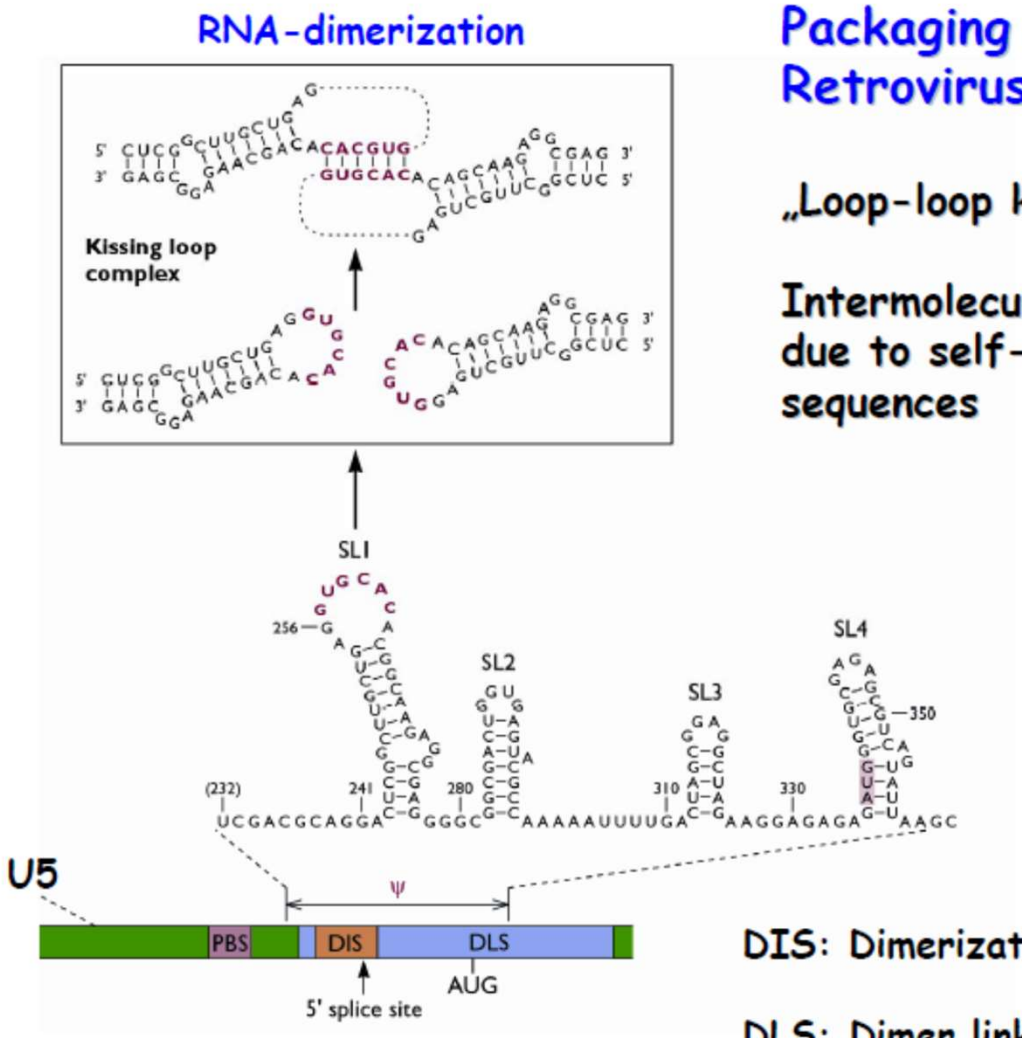
In vitro translation system to analyze capsid assembly



Detection of capsid assembly in cell free system



Packaging of the viral genome



Packaging of genomic RNA: Retroviruses

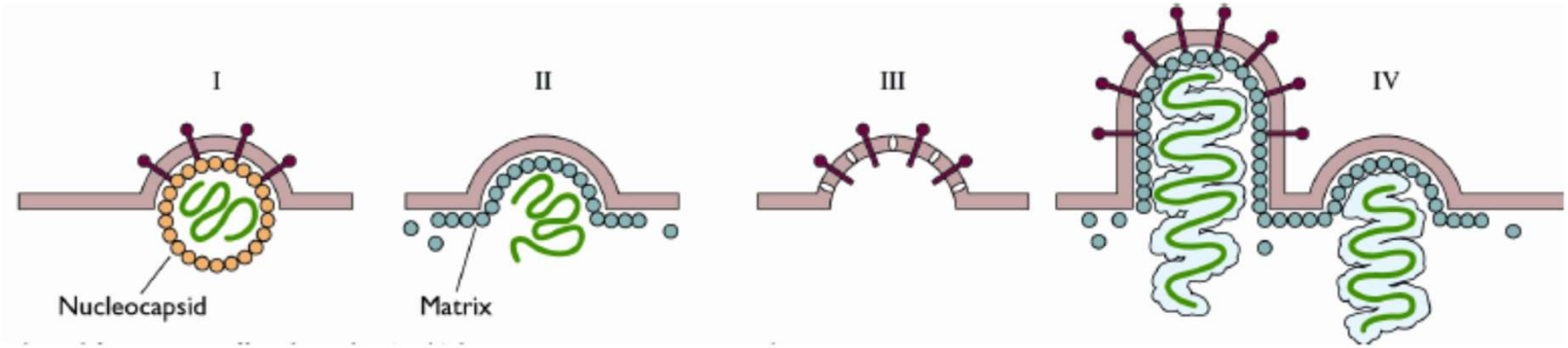
„Loop-loop kissing complex"

Intermolecular bonding due to self-complementary sequences

DIS: Dimerization initiation site

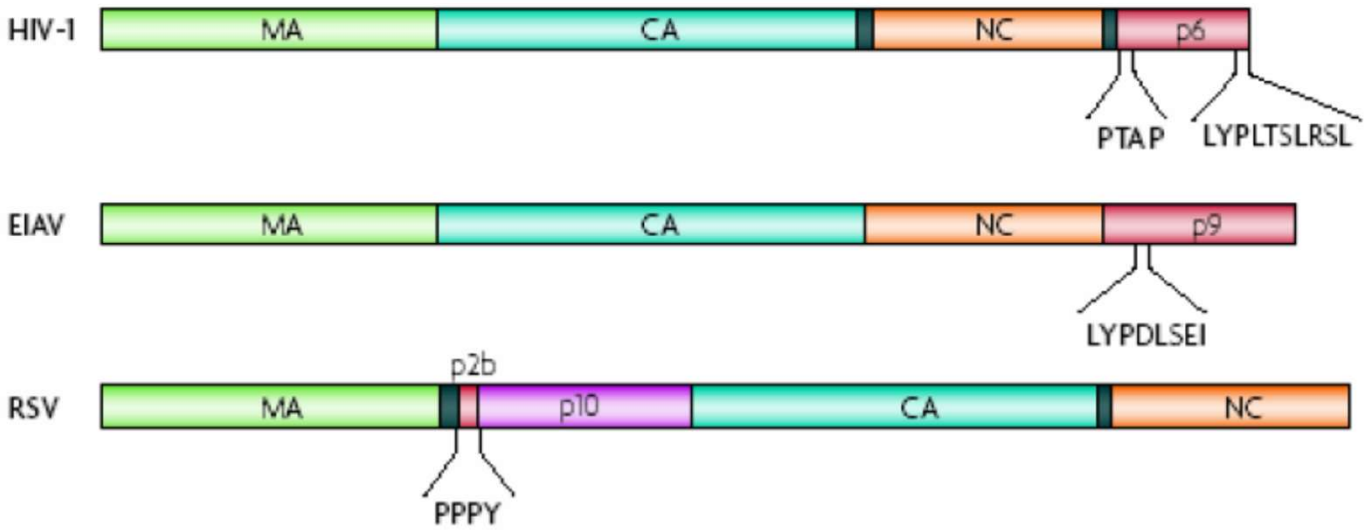
DLS: Dimer linkage sequence

Assembly and budding of envelope viruses



Env + CA required	M + CA + RNA required	Env sufficient	MA, RNP, ENV
Alphaviruses	Retroviruses	MHV	Orthomyxo

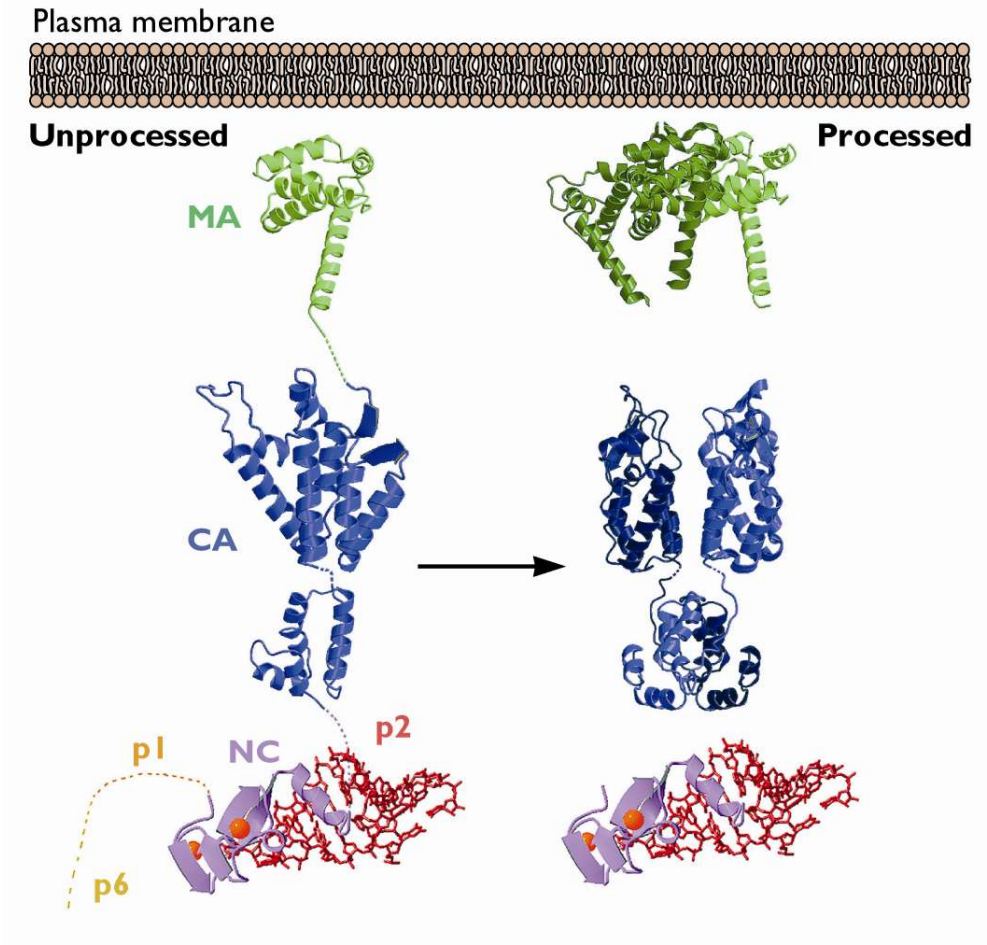
Retroviral Gag proteins contain late domain

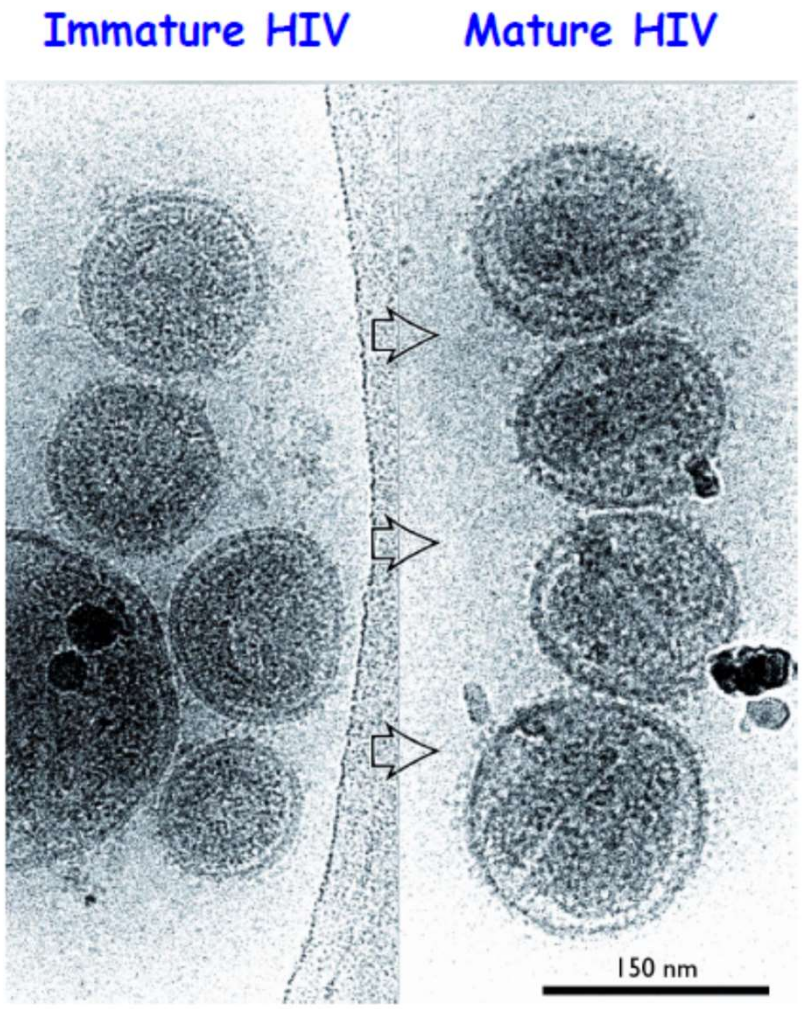


Late domains interact with vacuolar protein sorting (VPS) proteins.

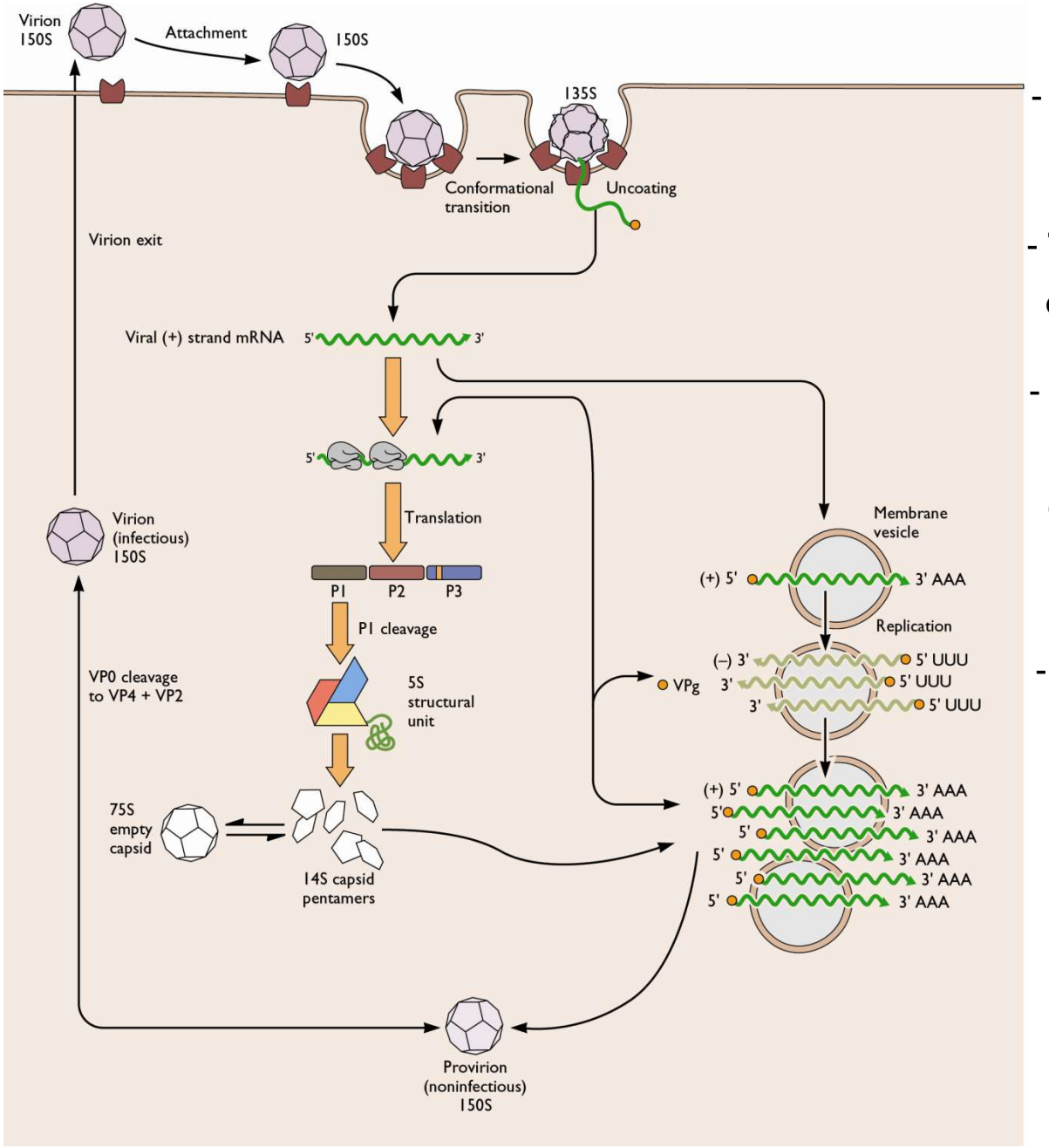
PTAP: Tsg101
LYPLTSLRSL/LYPDLSEI: Alix (apoptosis linked gene 2 interacting protein)
PPPY: Nedd4 ubiquitin ligases

Virus maturation





Poliovirus assembly



- formation of immature structure 5S subunit (VP0, VP3, VP1)

- 75S empty capsids storage forms of 14S pentamers

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

- VP0 cleavage to VP4 and VP2 infectious virion released

Adenovirus

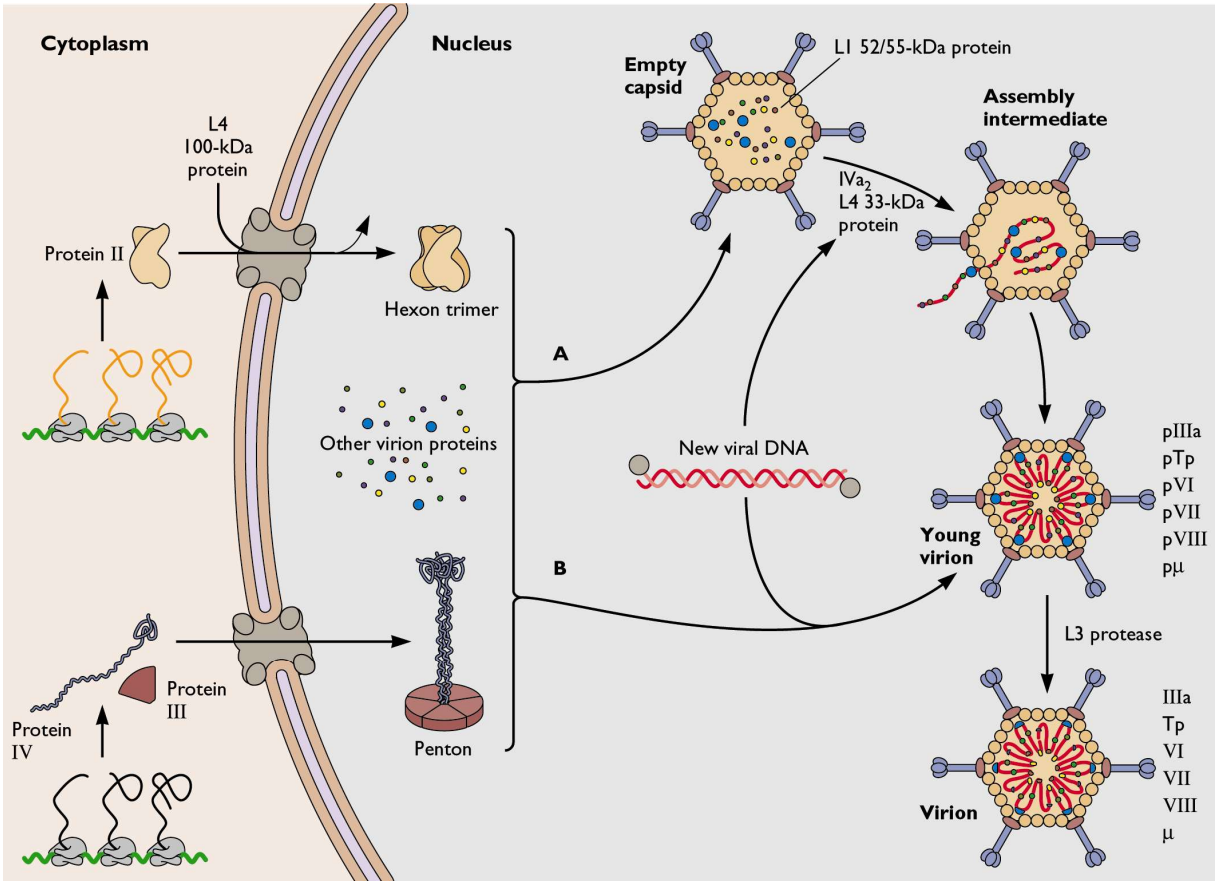
- synthesis and assembly of hexons and pentons, transport into nucleus

- L4 protein required for formation of hexons

- (A) structural units and proteins assemble into emtpy capsids

- IVa2 binds to packaging signal of genome: assembly intermediate

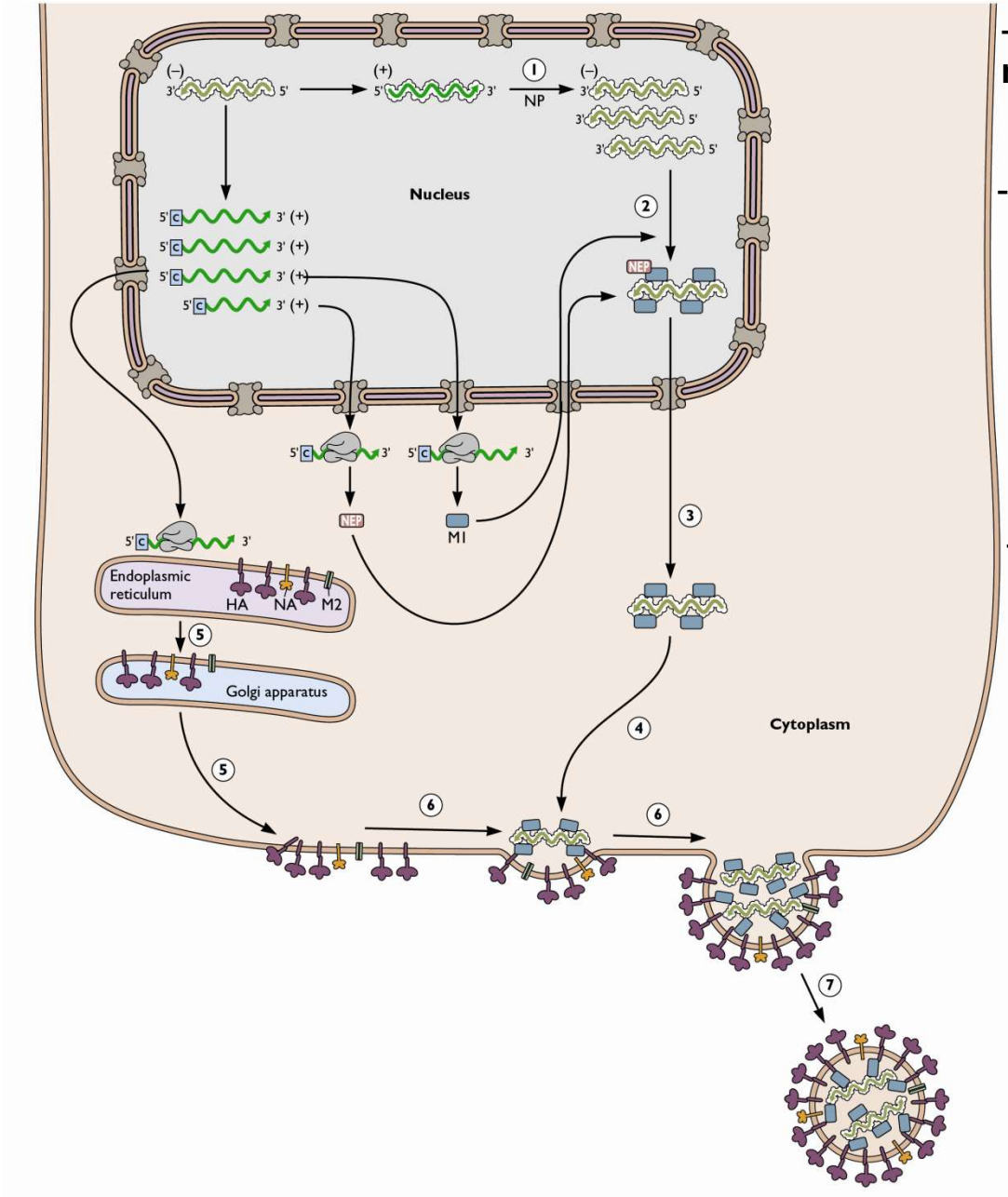
- Mature particles are produced upon cleavage of the precursor proteins



- (B) is based on the failure of any capsid-like structures to assemble (mutations)

- then capsid assembly and encapsidation of the genome are concerted reactions

Influenzavirus



- (-) strand genomic RNA synthesized in the nucleus

- packaging by the NP RNA-binding proteins

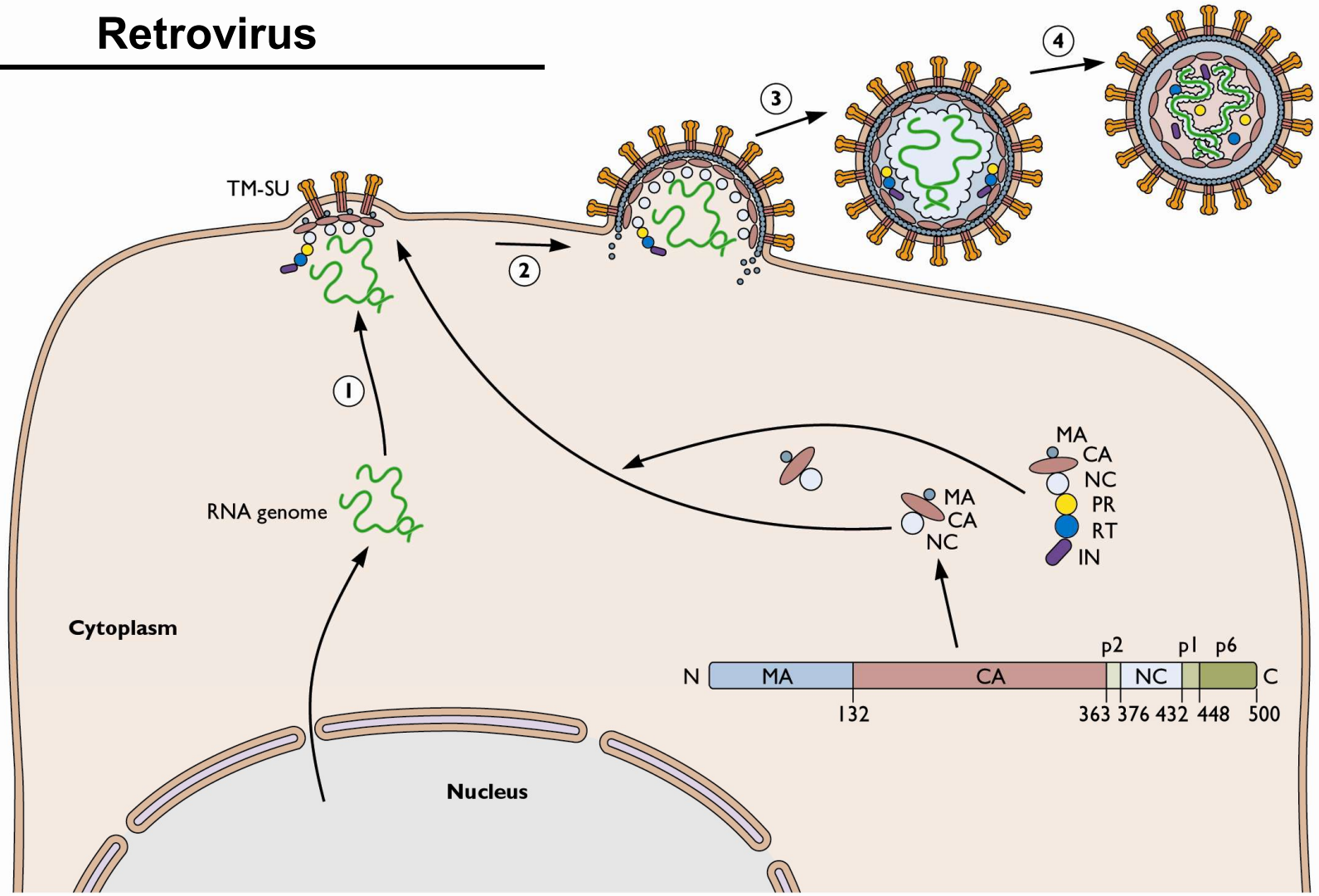
- binding to M1 protein prevents further transcrption or replication and allows binding of of NEP

- Export of nucleocapsid to the cytoplasma

- M1 also binds to PM which carries HA, NA and M2 proteins

- M1 controls budding

Retrovirus



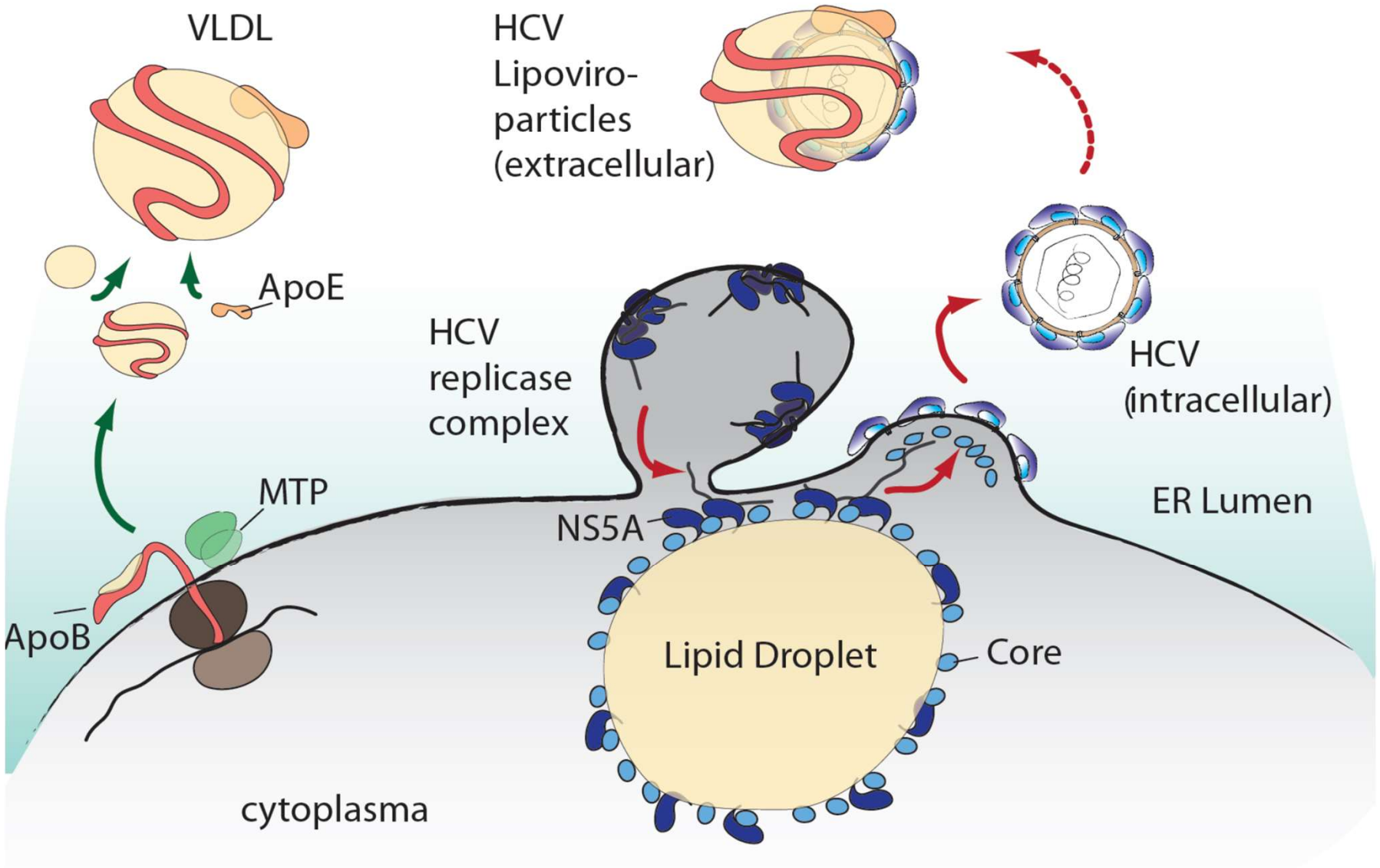
- gag polyprotein of all retroviruses contains MA, CA and NC proteins

- association of gag molecules at PM with one another and with the RNA genome initiated budding

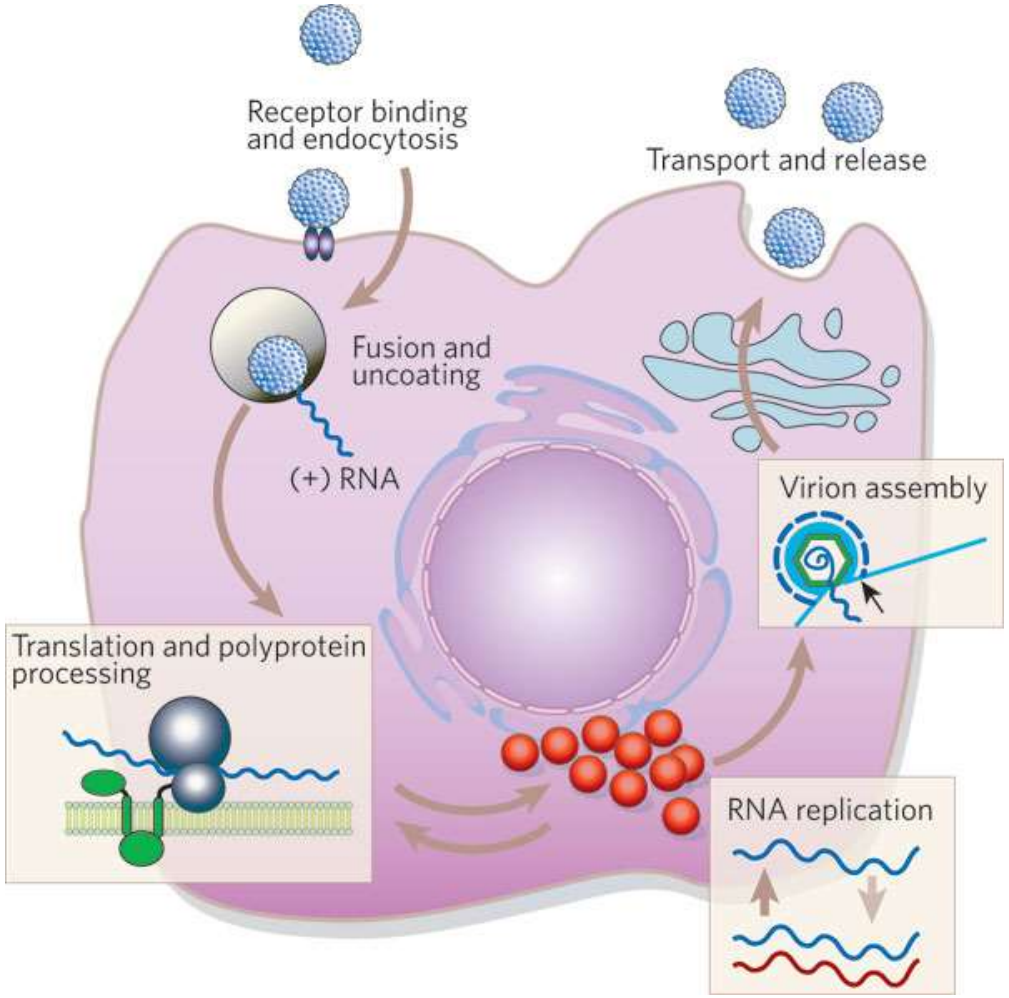
- incorporation of further gag molecules , release of of immature non-infectious particle

- cleavage of gag and gag-pol by PR produced infectious particles

HCV Morphogenesis



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