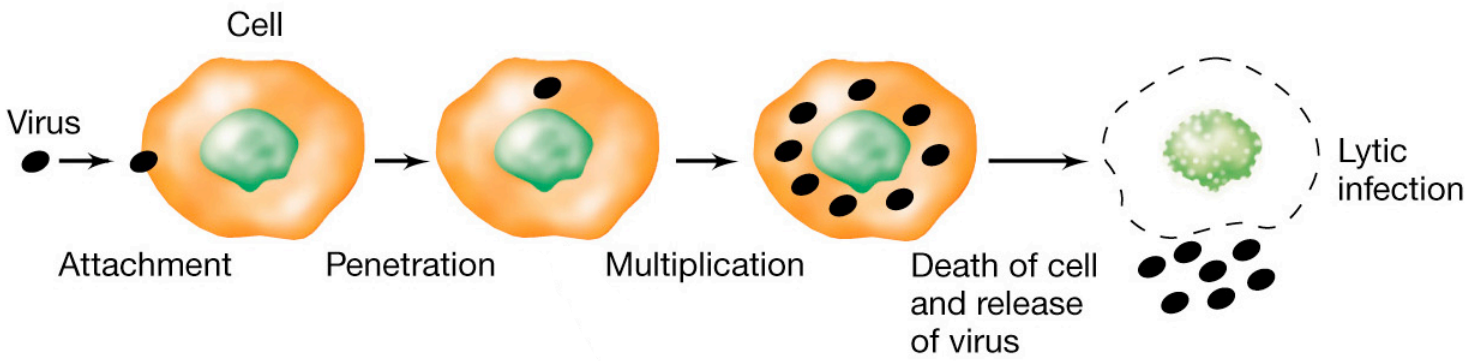
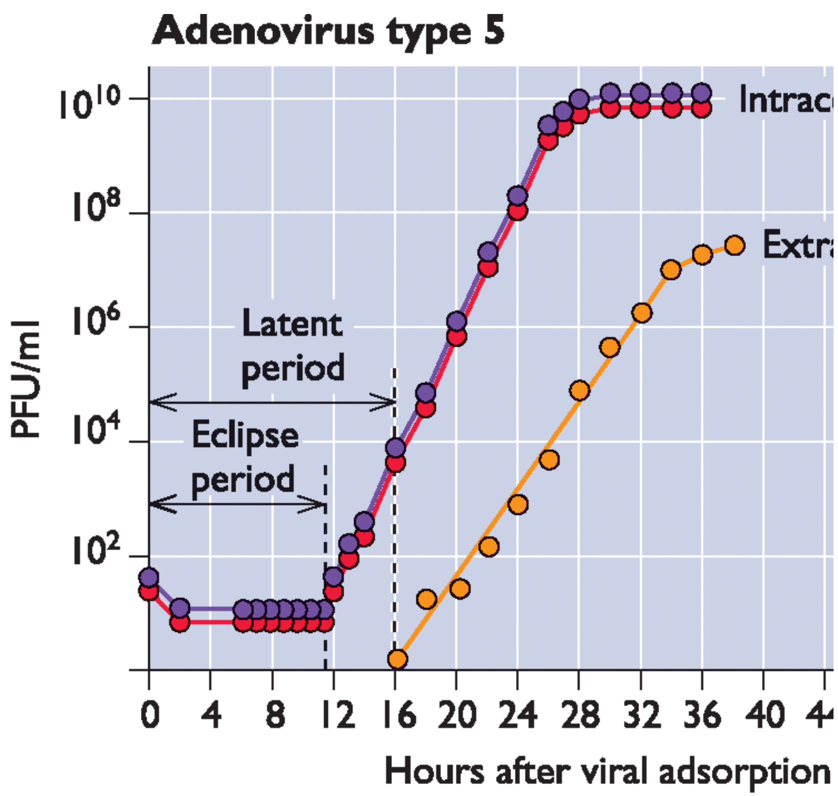
VIROLOGY

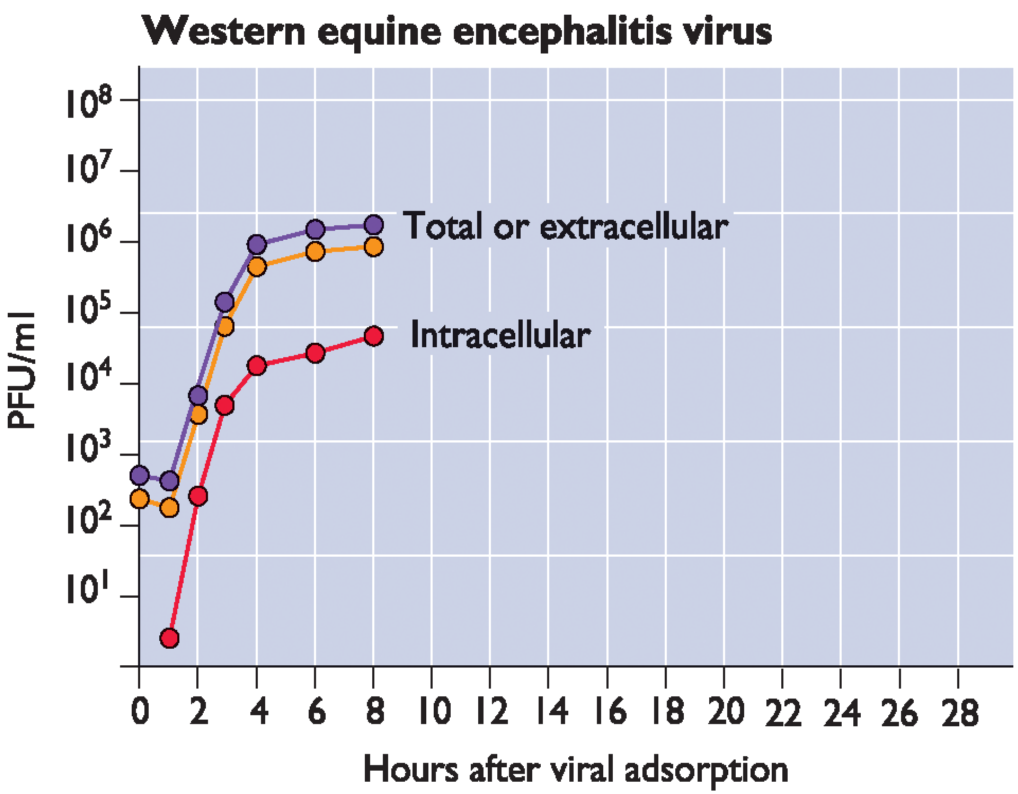
The infectious cycle



Possible cytopathogenesis of cells
infected with animal viruses

The one-step growth curve is a fundamental feature of a virus



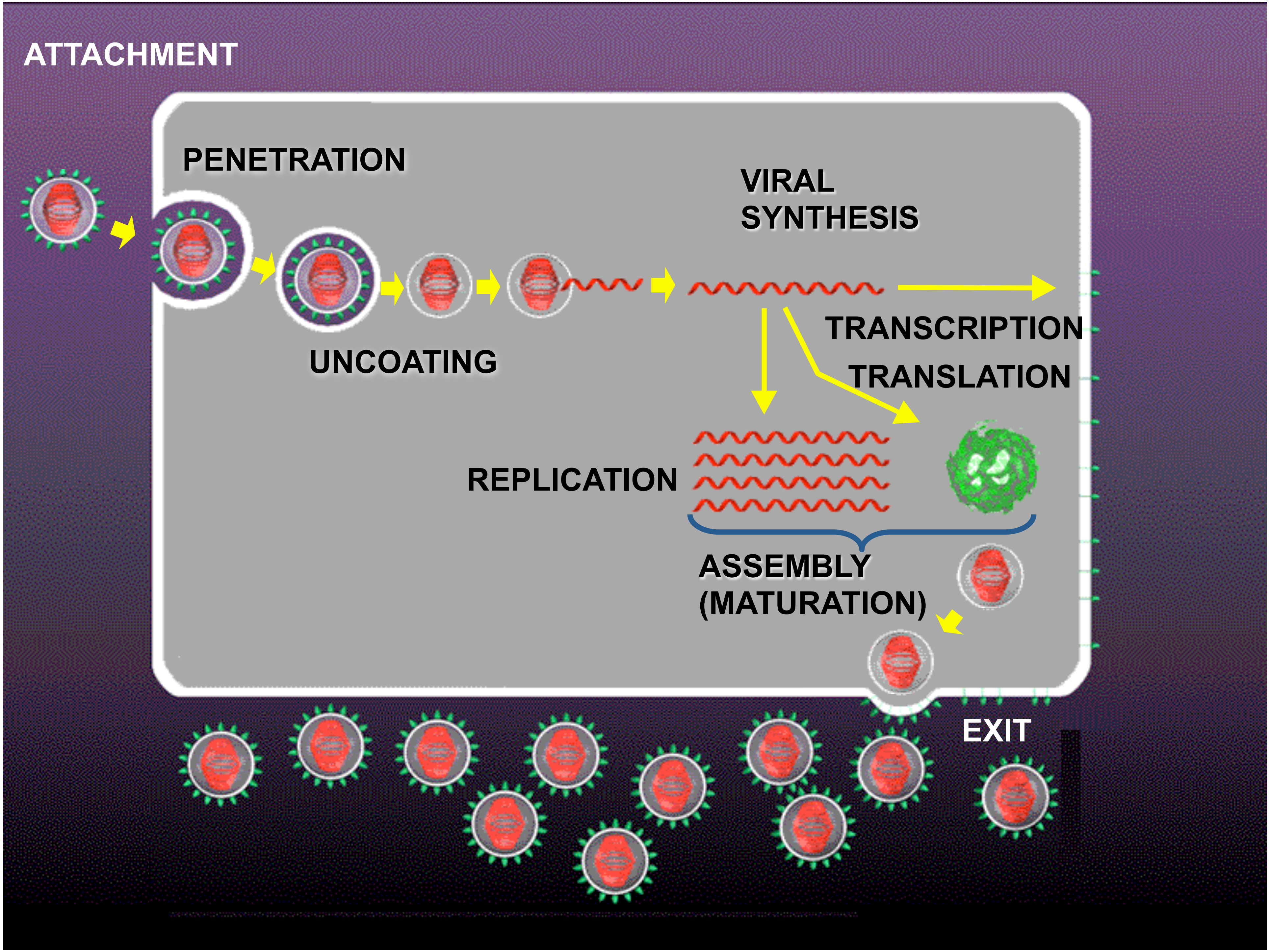


The time interval from infection to plateau represents the time required for a single cycle of growth.

The yield of virus at plateau shows the amount of virus produced per cell during a single round of infection .

The reproductive cycle of animal viruses

- Virus attachment to host cell
- Virus entry into cells
- Transcription, translation and genome replication
- Assembly, exit and maturation of progeny virions


ATTACHMENT
PENETRATION
VIRAL
SYNTHESIS
UNCOATING
TRANSCRIPTION
TRANSLATION
REPLICATION
ASSEMBLY
(MATURATION)
EXIT

The infectious cycle:

virus attachment to host cells

Viral receptors and coreceptors

Table 4.1 Viral receptors and coreceptors[a]

Virus	Receptor	Type of molecule	Coreceptor
Picornaviridae			
Foot-and-mouth disease virus (cell culture adapted)	Heparan sulfate	Glycosaminoglycan	
Foot-and-mouth disease virus	$\alpha_v\beta_3$ (vitronectin receptor)	Integrin	
Encephalomyocarditis virus	Vcam-1	Ig-like	
	Sialylated glycophorin A (for hemagglutination only)	Carbohydrate	
Poliovirus type 1 to 3	Pvr	Ig-like	
Coxsackieviruses A13, A18, A21	Icam-1	Ig-like	
Coxsackievirus A21	Decay-accelerating protein (CD55)	SCR-like (complement cascade)	Icam-1
Coxsackievirus A9	$\alpha_v\beta_3$	Integrin	
Coxsackieviruses B1 to B6	Car (coxsackievirus-adenovirus receptor)	Ig-like	
Coxsackieviruses B1, B3, B5	CD55	SCR-like (complement cascade)	$\alpha_v\beta_6$ integrin
Echoviruses 1 and 8	$\alpha_2\beta_1$ integrin (Vla-2)	Integrin	β_2 microglobulin
Echovirus 22	$\alpha_v\beta_3$ (vitronectin receptor)	Integrin	
Echoviruses 3, 6, 7, 11 to 13, 20, 21, 24, 29, 33	CD55	SCR-like (complement cascade)	β_2 microglobulin
Enterovirus 70	CD55	SCR-like (complement cascade)	
Bovine enterovirus	Sialic acid	Carbohydrate	
Hepatitis A virus	HAVCr-1	Ig-like, mucin-like	
Major group rhinoviruses (91 serotypes)	Icam-1	Ig-like	
Minor group rhinovirus (10 serotypes)	Low-density lipoprotein receptor protein family	Signaling receptor	
Rhinovirus 87	Sialic acid	Carbohydrate	
Coronaviridae			
Mouse hepatitis virus	Bgp (biliary glycoprotein)	Ig-like	
Human coronavirus 229E	Aminopeptidase N	Protease	
Transmissible gastroenteritis virus	Aminopeptidase N	Protease	
Human coronavirus OC43	Sialic acid	Carbohydrate	
Bovine coronavirus	Sialic acid	Carbohydrate	
Togaviridae			
Semliki Forest virus	Major histocompatibility class I molecule	Ig-like	
Sindbis virus	High-affinity laminin receptor	Integrin	
	Heparan sulfate	Glycosaminoglycan	
Dengue virus	Heparan sulfate	Glycosaminoglycan	
Rhabdoviridae			
Rabies virus	Nicotinic acetylcholine receptor	Neurotransmitter receptor	
	Neural cell adhesion molecule CD56	Ig-like	
	Low-affinity nerve growth factor receptor	Tnf receptor protein superfamily	
Paramyxoviridae			
Measles virus	Membrane cofactor protein, CD46	Complement-regulating protein	
Sendai virus	Sialic acid	Carbohydrate	
	Asialoglycoprotein receptor Gp-2	Transport protein (receptor-mediated endocytosis)	
Orthomyxoviridae			
Influenza A and B viruses	Sialic acids (*N*-acetyl neuraminic acid)	Carbohydrate	
Influenza C virus	Sialic acids (9-*O*-acetyl neuraminic acid)	Carbohydrate	
Arenaviridae			
Lymphocytic choriomeningitis virus	α-Dystroglycan	Laminin receptor	
Lassa virus	α-Dystroglycan	Laminin receptor	

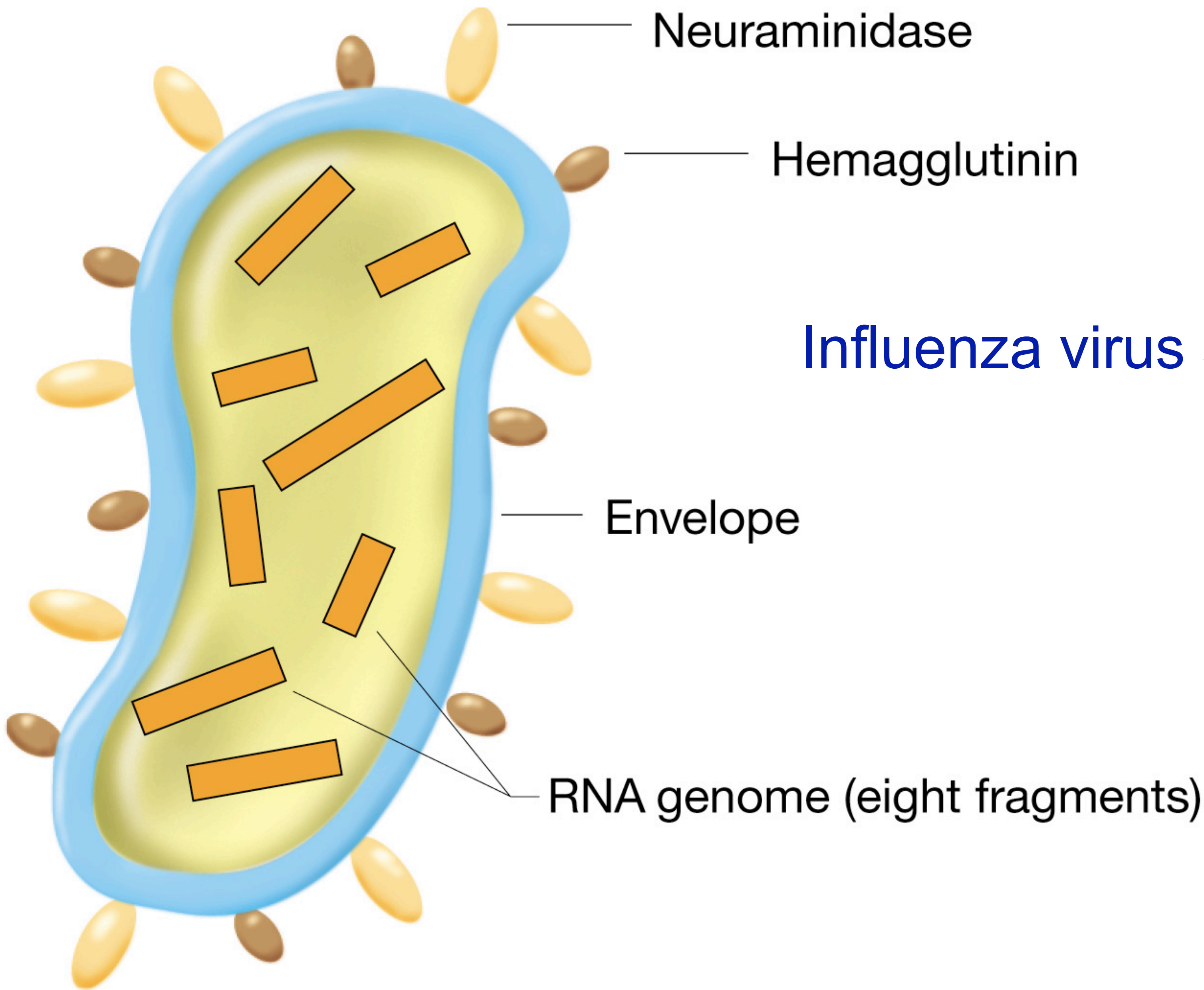
(contin

Table 4.1 Viral receptors and coreceptors[a] *(continued)*

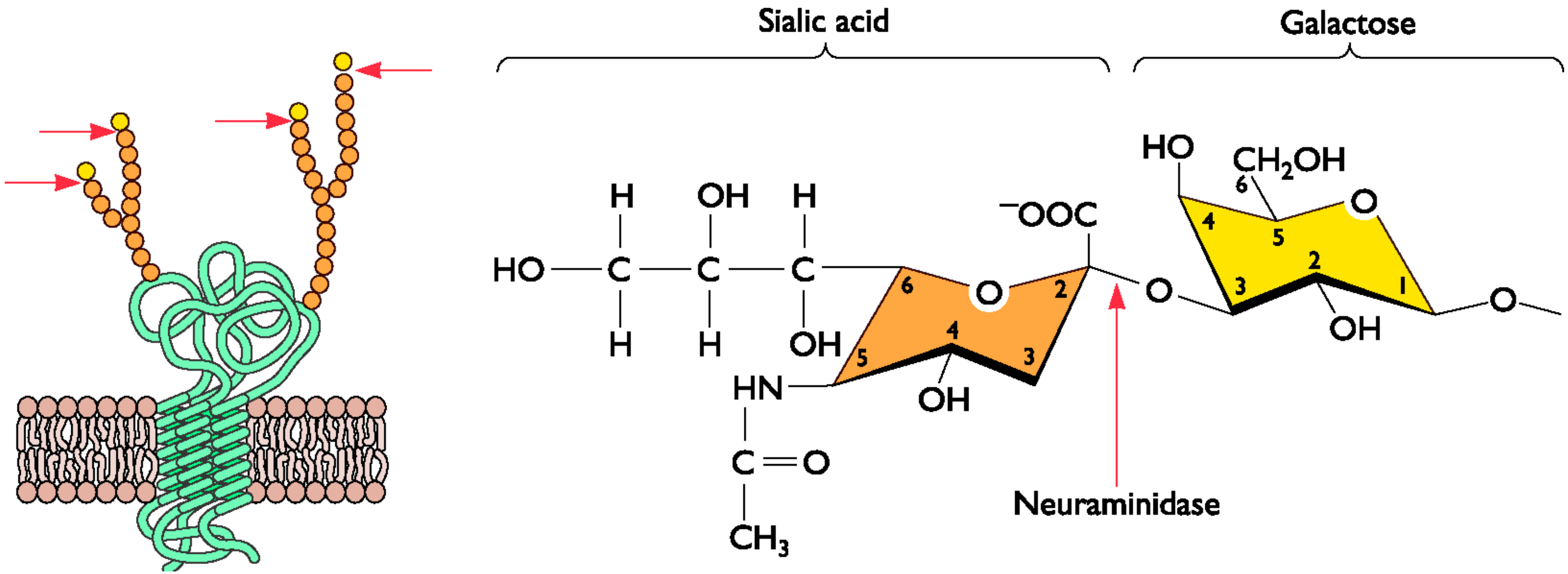
Virus	Receptor	Type of molecule	Coreceptor
Reoviridae			
Reovirus	Sialic acids	Carbohydrate	
Group A porcine rotavirus	Sialic acids	Carbohydrate	
Retroviridae			
Human immunodeficiency virus type 1	CD4	Ig-like	Chemokine receptors (Ccr5, Cxcr4, Ccr3)
	Galactosylceramide	Glycolipid	
Human immunodeficiency virus type 2	CD4	Ig-like	Chemokine receptors
	Cxcr4	7-transmembrane superfamily	
Simian immunodeficiency virus	CD4	Ig-like	Chemokine receptors
Gibbon ape leukemia virus	Glvr1	Sodium-dependent phosphate transport protein	
Feline leukemia virus B	Glvr1	Sodium-dependent phosphate transport protein	
Amphotropic murine leukemia virus	Ram-1	Sodium-dependent phosphate transport protein	
Ecotropic murine leukemia virus	Cat	Cationic amino acid transport protein	
Subgroup A avian leukosis and sarcoma virus	Tva	Low-density lipoprotein receptor protein family	
Subgroup B and D avian leukosis and sarcoma viruses	Car1	Tnf receptor family protein superfamily	
Bovine leukemia virus	BLVRcp 1	Unknown	
Feline immunodeficiency virus	Cxcr4	7-transmembrane superfamily	
Visna virus	Major histocompatibility complex class II molecule	Ig-like	
Parvoviridae			
Bovine parvovirus	Sialic acids	Carbohydrate	
Adeno-associated virus type 2	Heparan sulfate	Glycosaminoglycan	$\alpha_v\beta_5$ integrin
Papovaviridae			
Simian virus 40	Major histocompatibility class I molecule	Ig-like	
Adenoviridae			
Adenovirus subgroups A, C, D, E, F	Car	Ig-like	α_v integrins
Adenovirus type 5 (subgroup C)	Major histocompatibility class II molecule	Ig-like	α_v integrins
Adenovirus type 2 (subgroup C)	$\alpha_M\beta_2$	Integrin	α_v integrins
Adenovirus type 9 (subgroup D)	α_v integrins	Integrin	
Herpesviridae			
Herpes simplex type 1	Heparan sulfate	Glycosaminoglycan	HveA, Prr1
Herpes simplex type 2	Heparan sulfate	Glycosaminoglycan	HveA, Prr1, Prr2
Pseudorabies virus	Heparan sulfate	Glycosaminoglycan	Pvr, Prr1, Prr2
Bovine herpesvirus 1	Heparan sulfate	Glycosaminoglycan	Pvr, Prr1
Human herpesvirus 7	CD4	Ig-like	
Epstein-Barr virus	Complement receptor Cr2 (CD21)	SCR-like (complement cascade)	
Human cytomegalovirus	Heparan sulfate	Glycosaminoglycan	Aminopeptidase N (CD13)
Poxviridae			
Vaccinia virus	Heparan sulfate	Glycosaminoglycan	
	Epidermal growth factor receptor	Signaling receptor	

[a]The name of the receptor and the type of molecule are listed for selected viruses. When coreceptors have been identified, they are listed; a blank in the coreceptor column indicates that none have been identified to date. Abbreviations: Vcam, vascular cell adhesion molecule; Prr1, Prr2, Pvr-related proteins 1 and 2; SCR, short consensus repeat; Ig, immunoglobulin; Tnf, tumor necrosis factor; Car1, cytopathic avian leukosis and sarcoma virus receptor; Car, coxsackievirus and adenovirus receptor; HveA, herpesvirus entry mediator.

Influenza virus structure



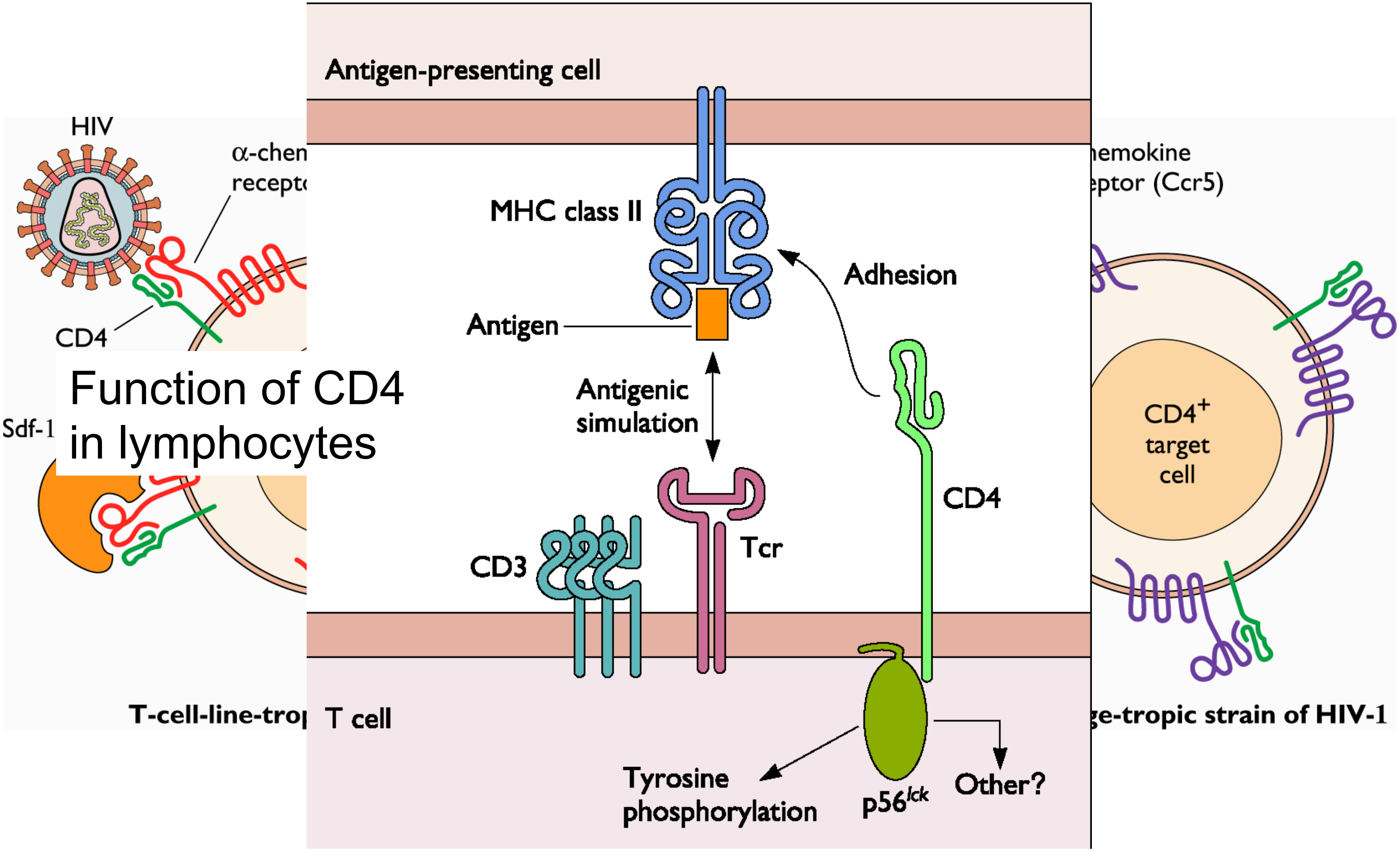
Sialic acid receptors for influenza virus

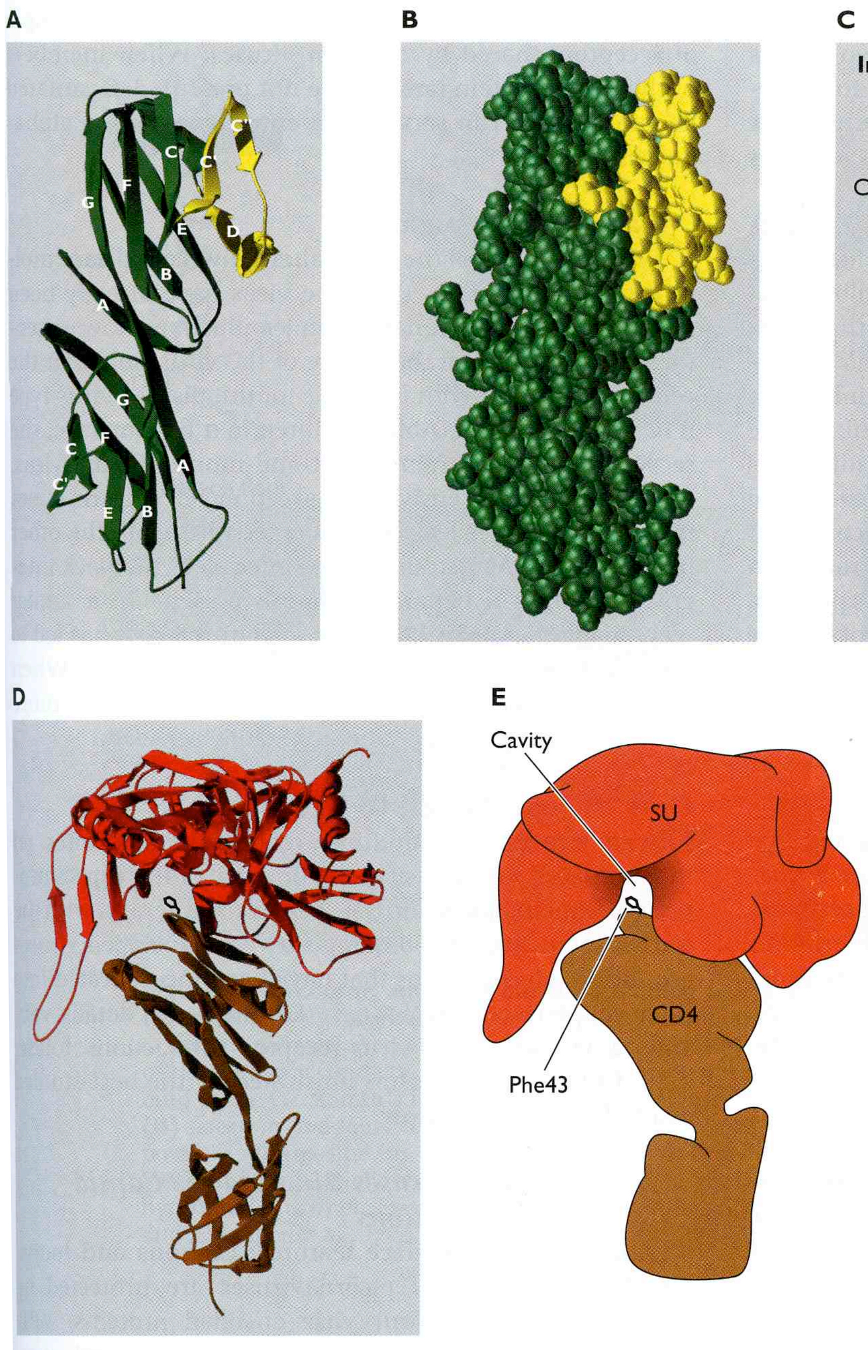


The interaction of influenza virus with sialic acid moieties is mediated by the viral surface glycoprotein **hemagglutinin** (**HA**)

Receptor and coreceptors for macrophage/monocyte- and T-cell- tropic strains of HIV-1

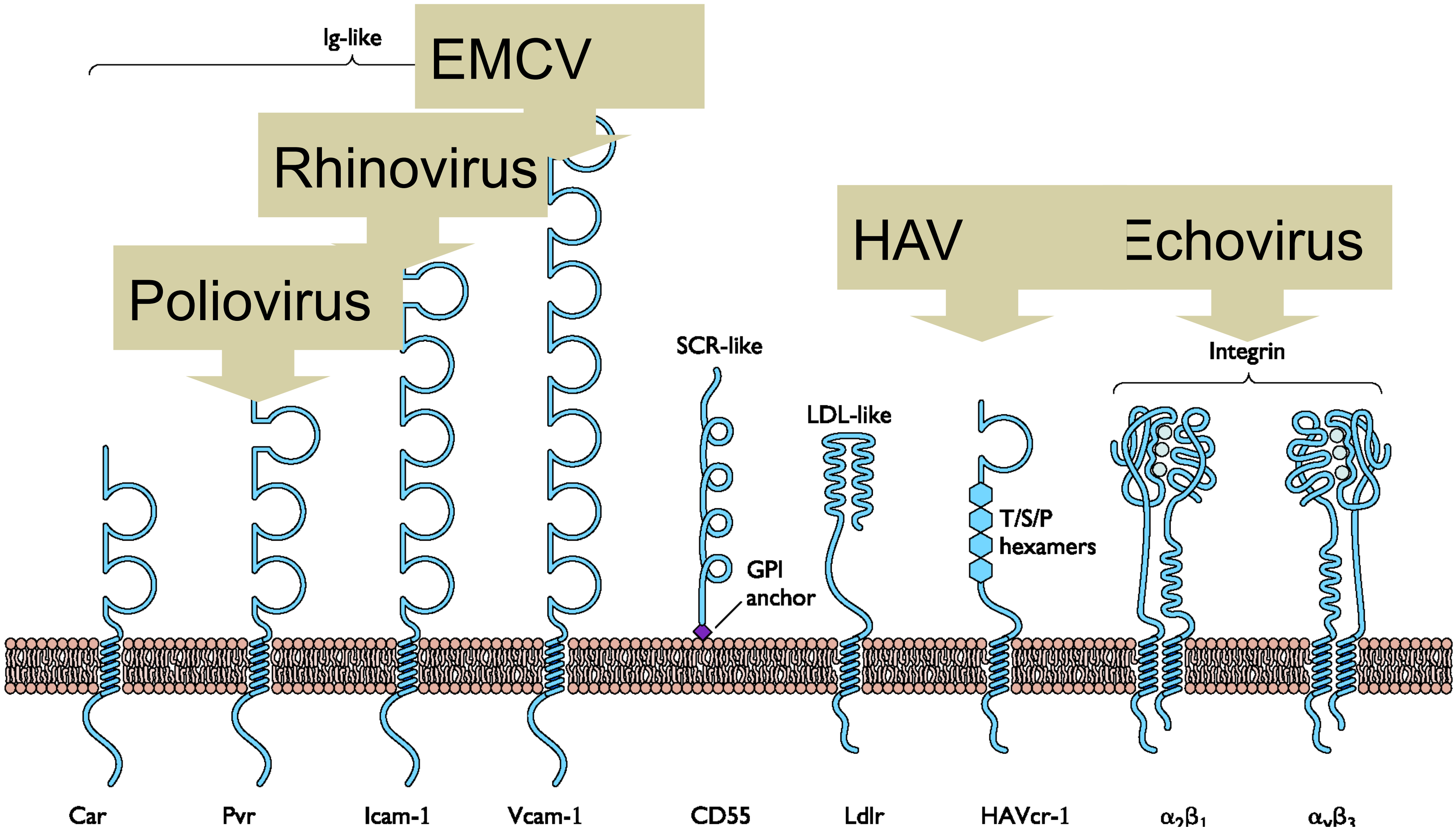
Function of CD4 in lymphocytes



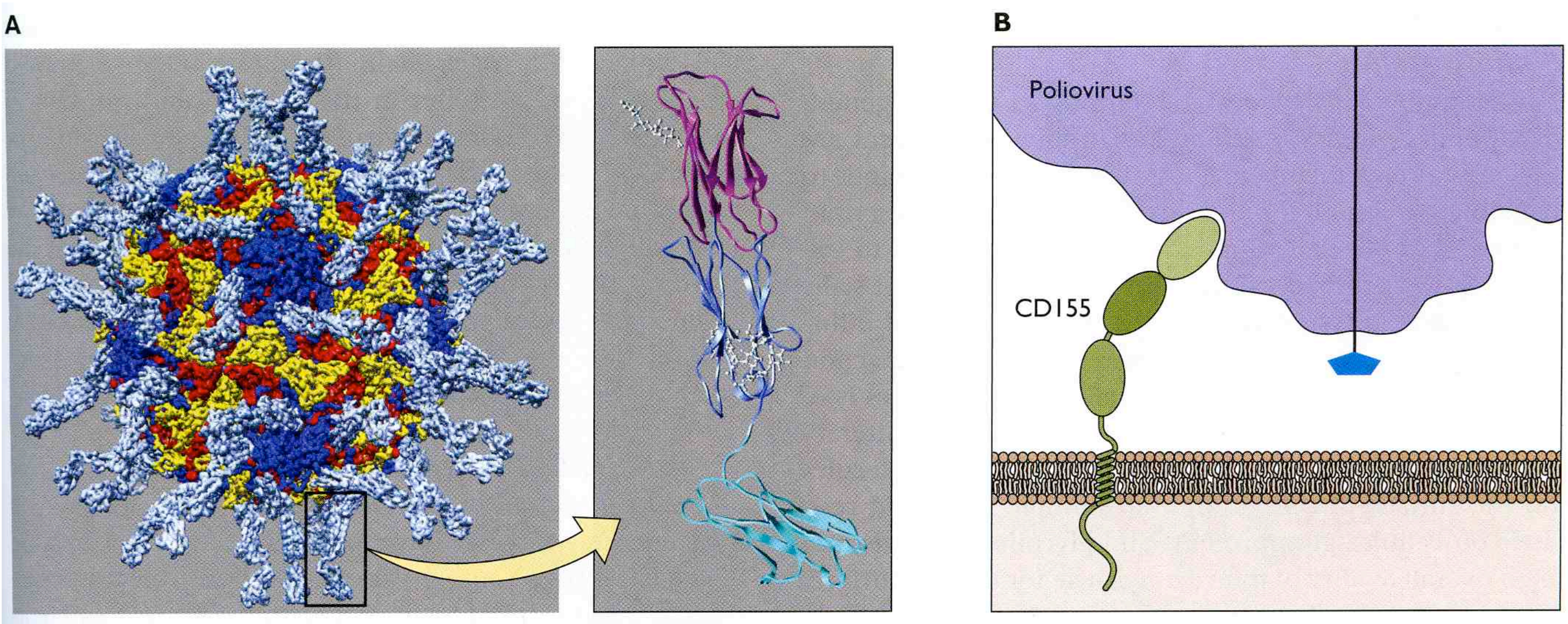


Interaction of HIV-1 SU with its cell receptor, CD4

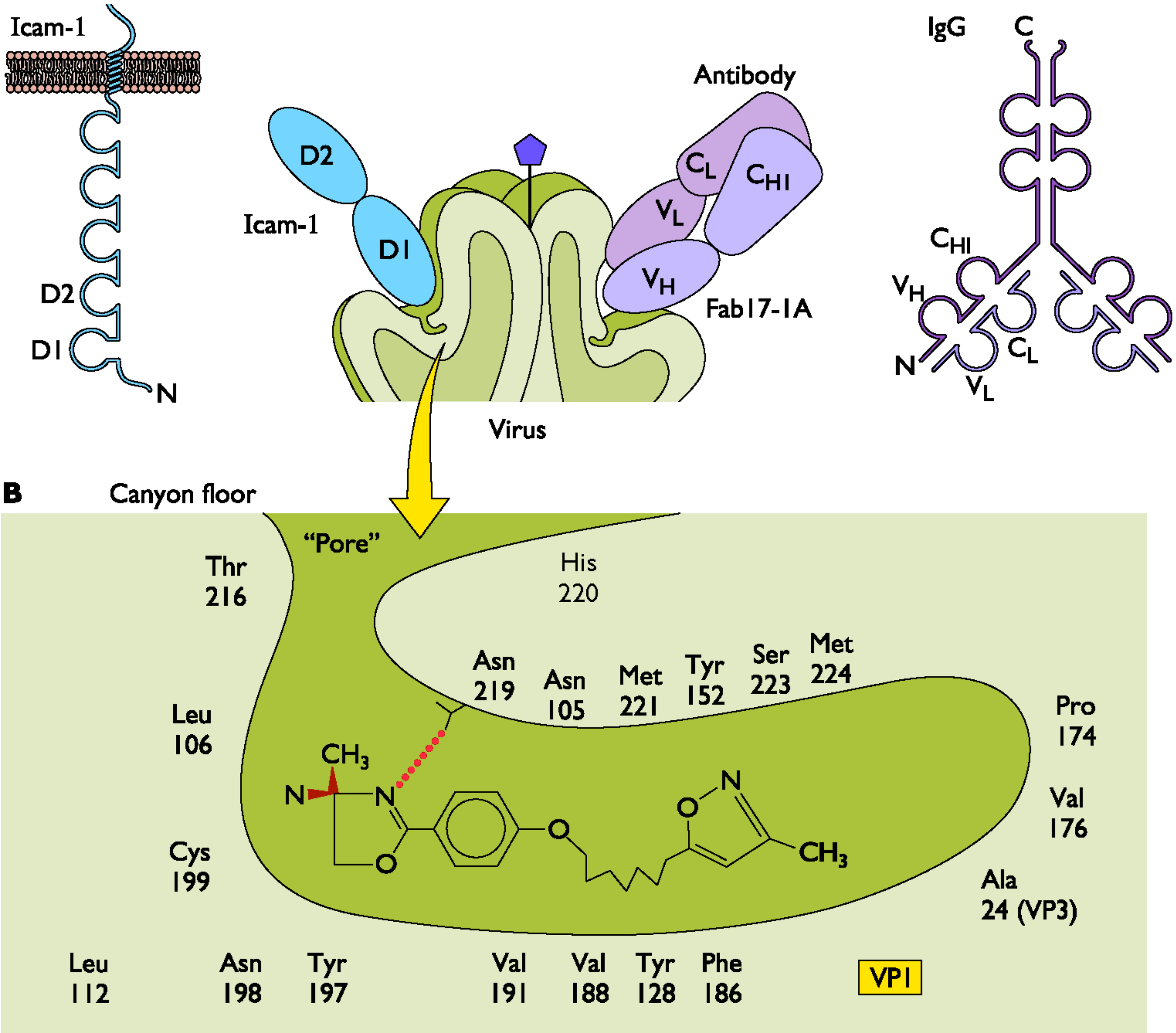
Cell receptors for picornaviruses



Poliovirus-receptor interactions



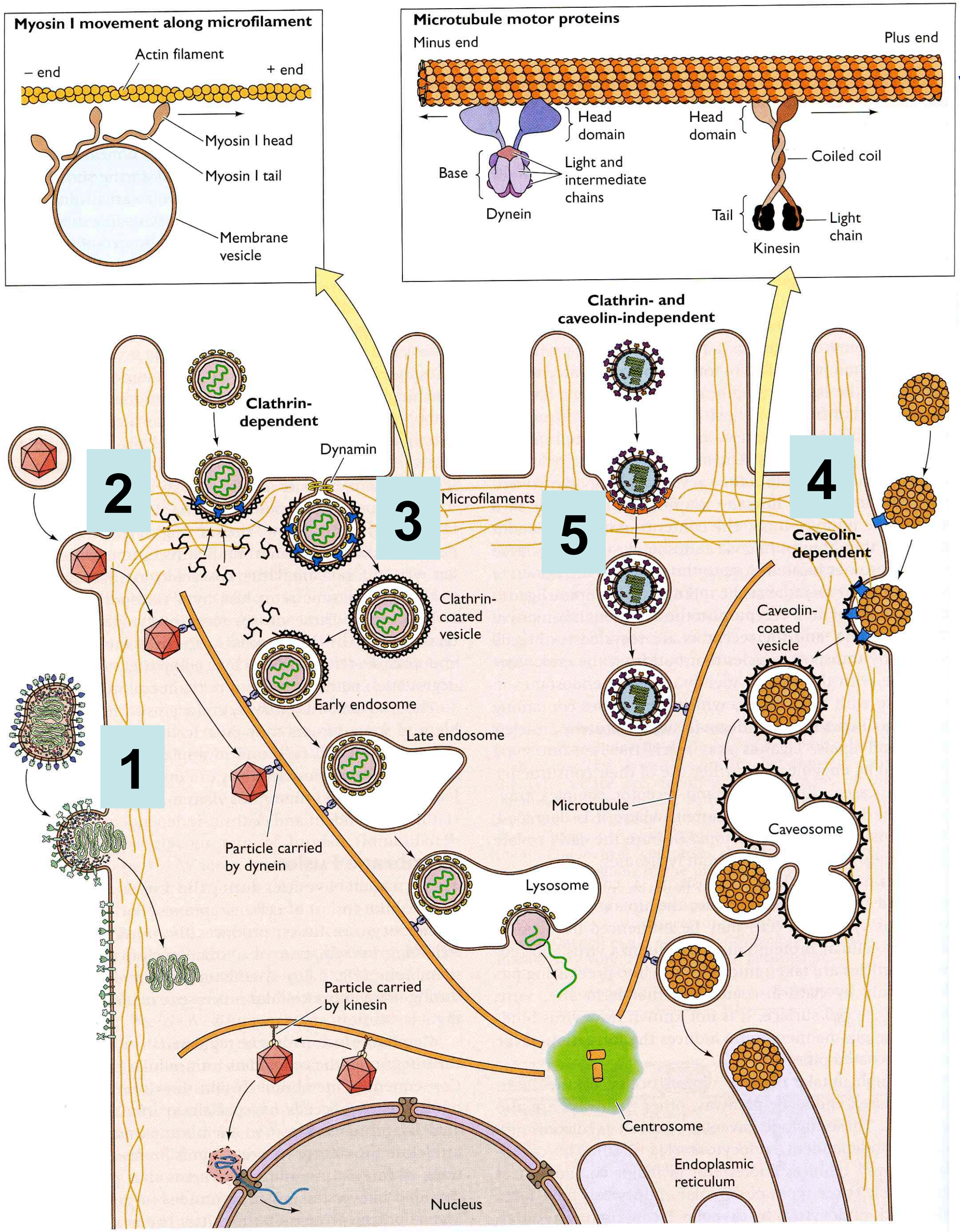
Receptor, antibody, and drug binding to the rhinovirus capsid



The infectious cycle:

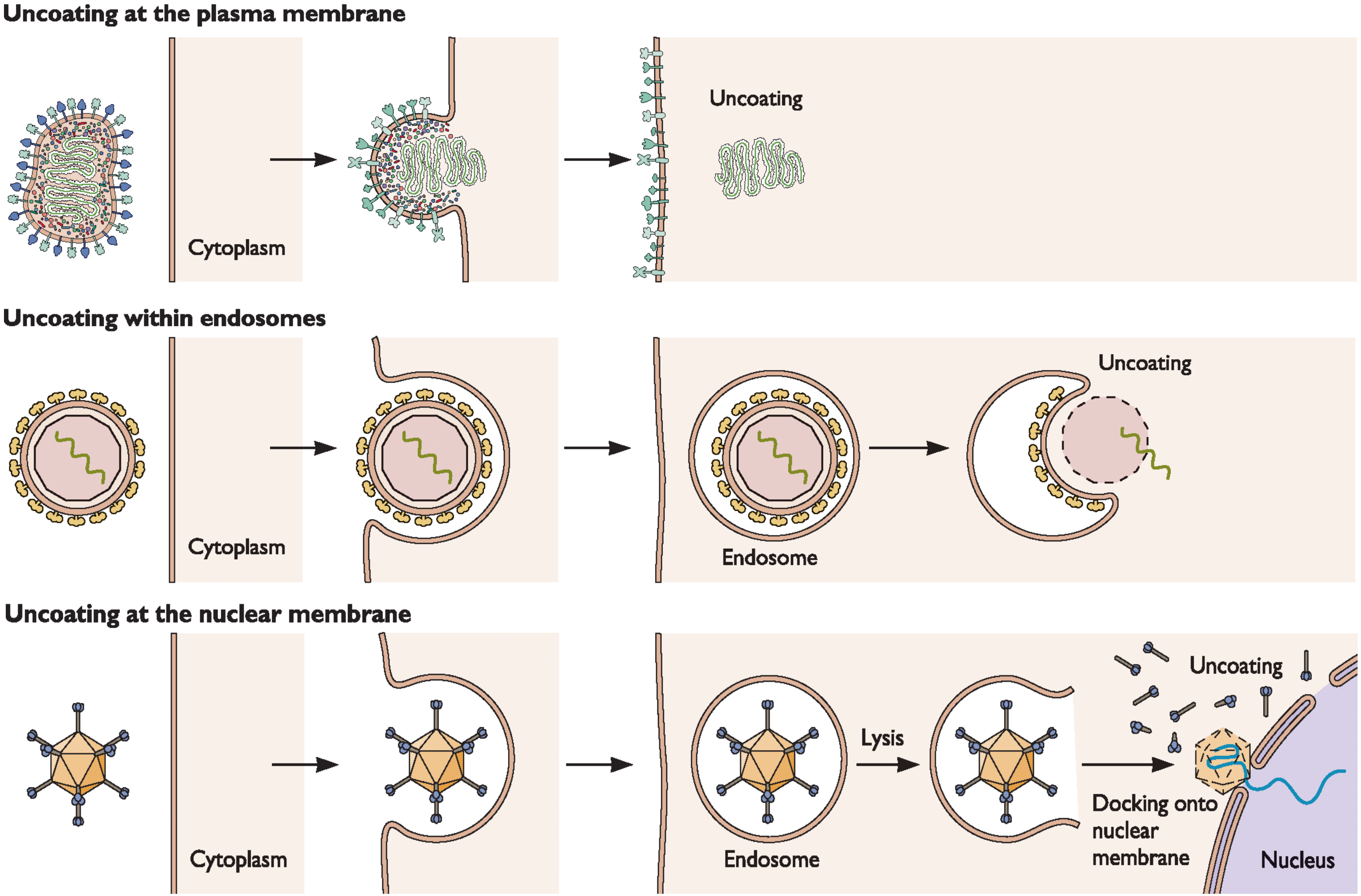
virus entry into host cells

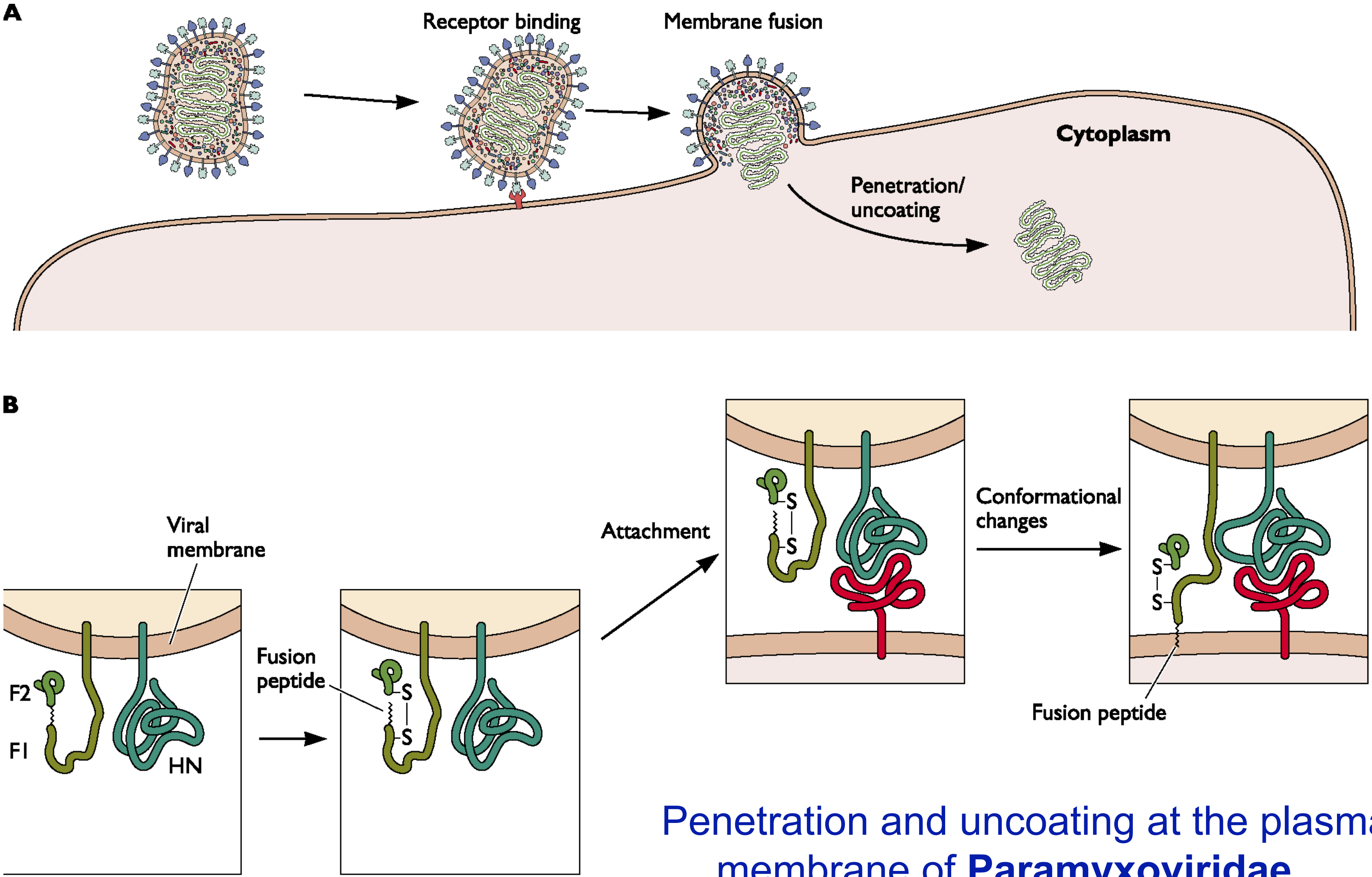
Virus entry strategies



1. Entry and uncoating at the **plasma membrane**
2. Entry at the **plasma membrane** and uncoating at the **nuclear membrane**
3. Entry by **clathrin-dependent** endocytosis
4. Entry by **caveolin-dependent** endocytosis (raft-mediated)
5. Entry **clathrin- and caveolin-independent** endocytosis

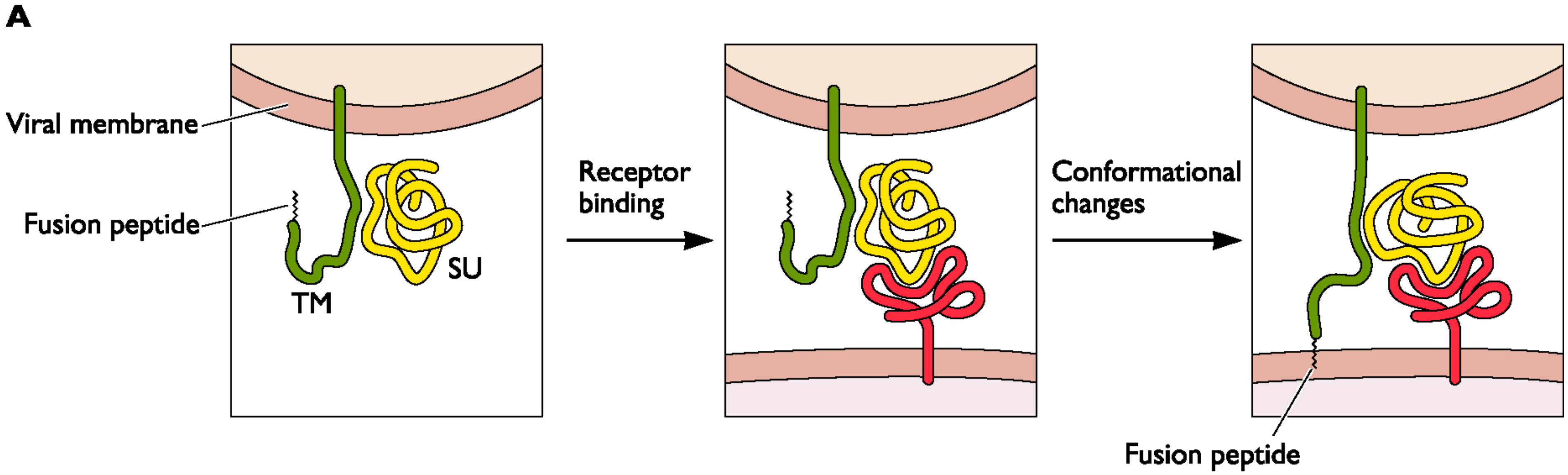
Three entry and uncoating strategies



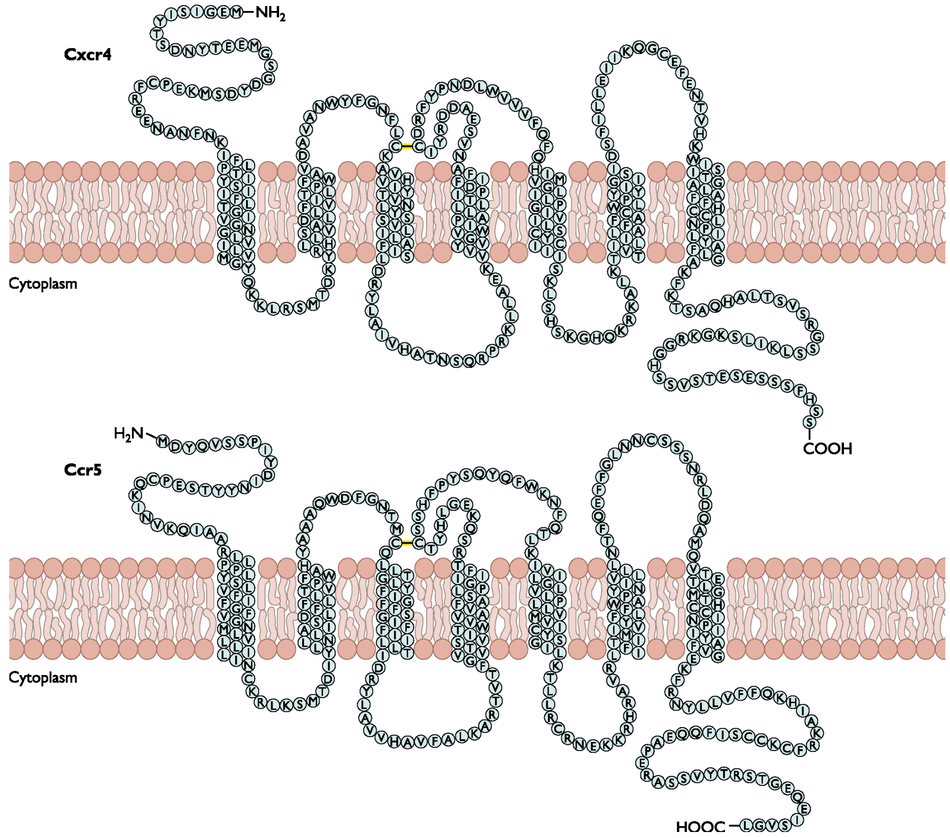


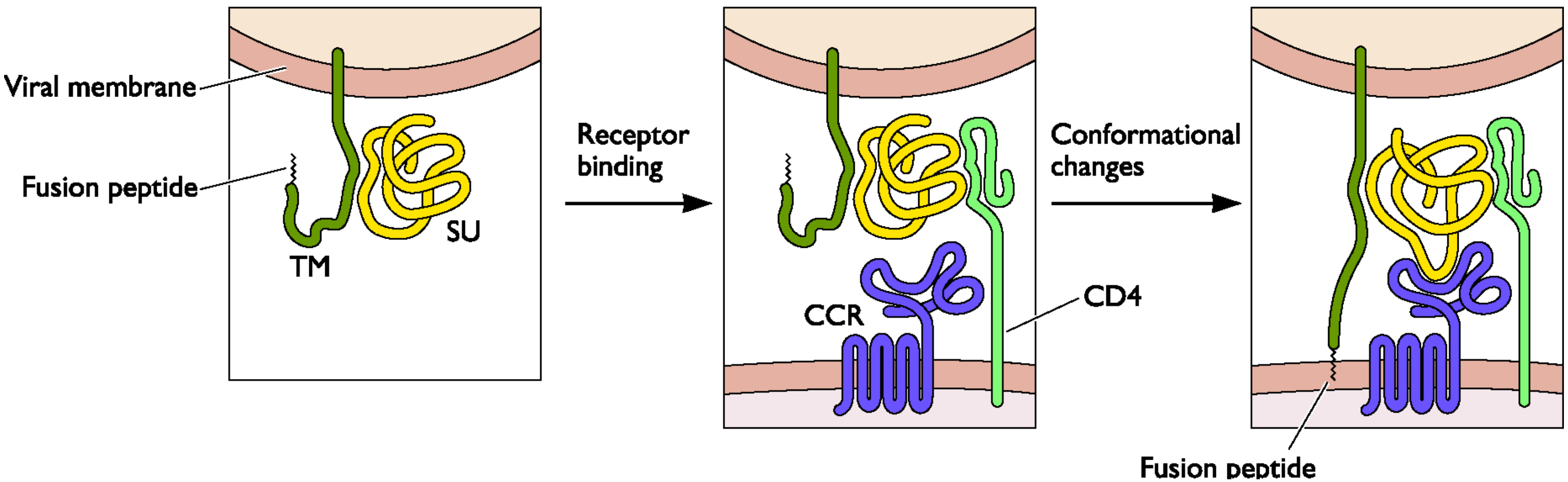
Penetration and uncoating at the plasma membrane of **Paramyxoviridae**

Mechanism of retroviral fusion with the plasma membrane

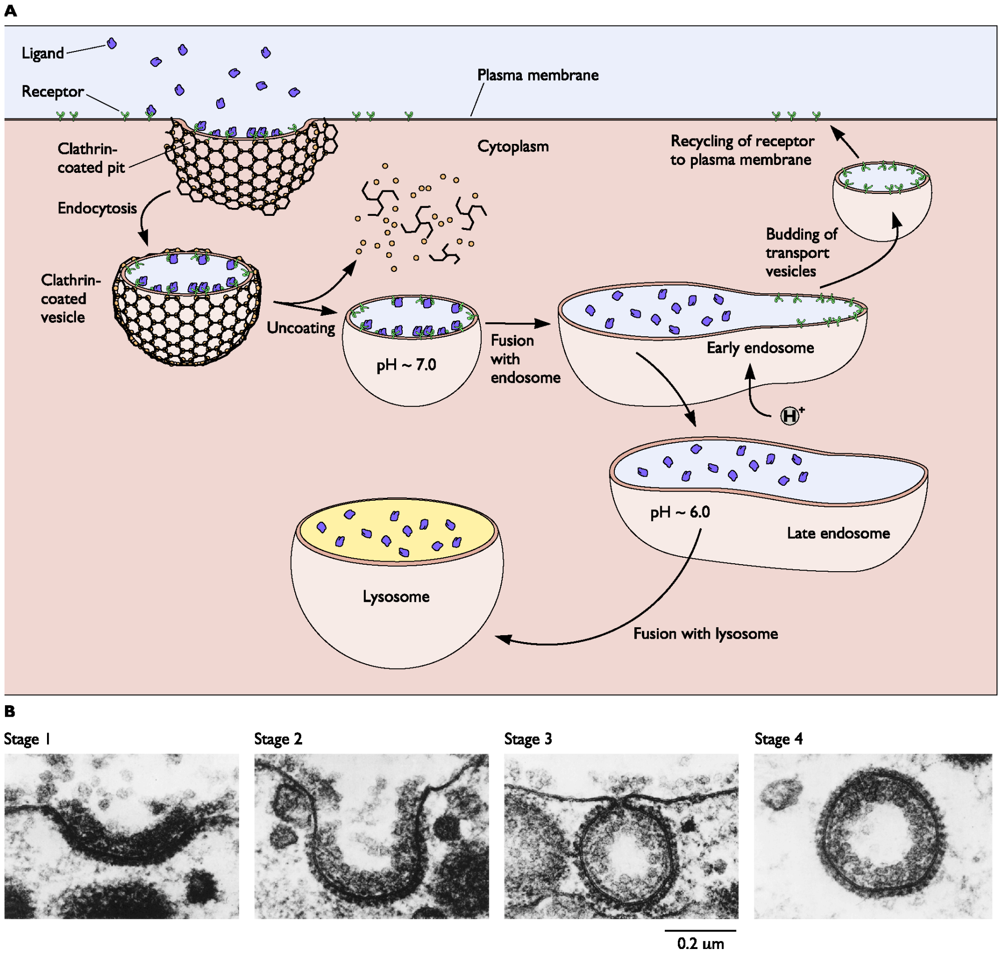


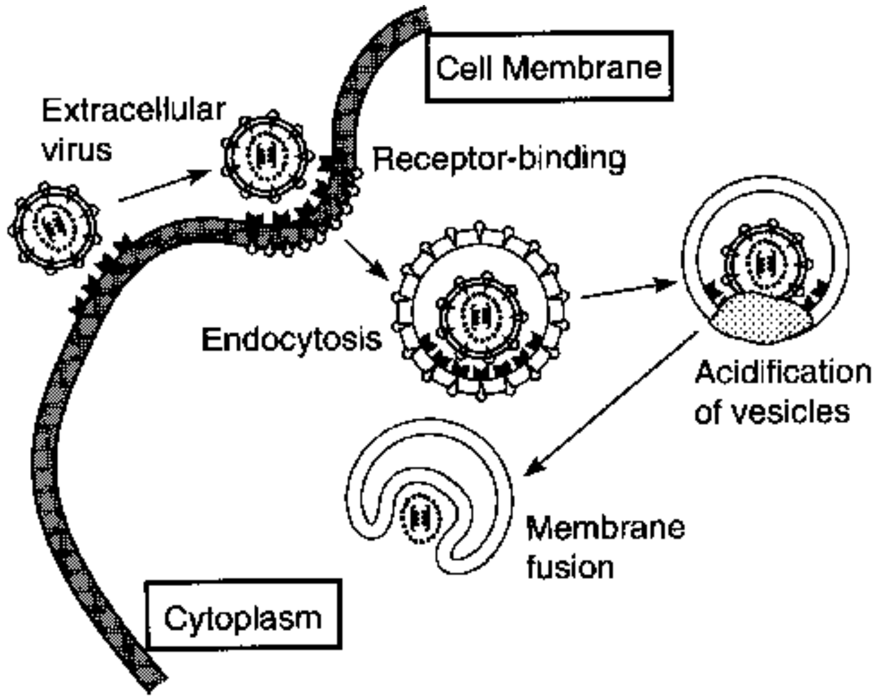
Mechanism of HIV-1 fusion with the plasma membrane



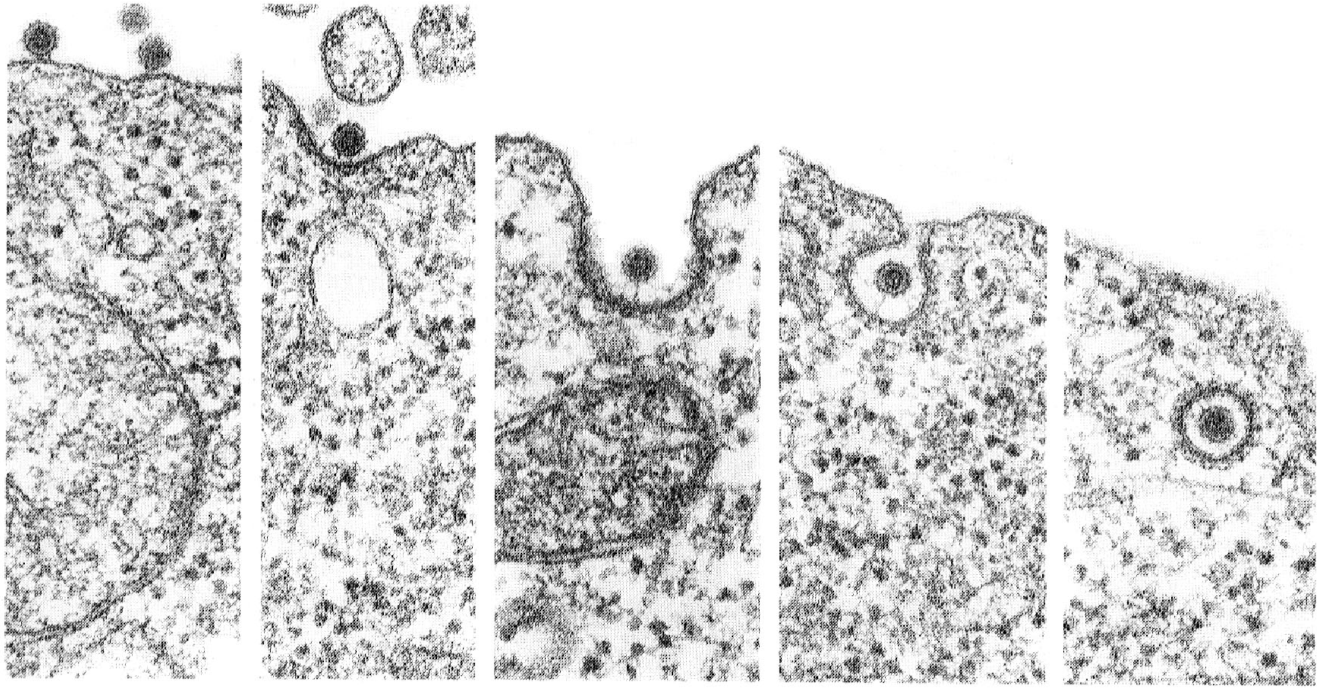


Mechanism of uncoating within endosomes

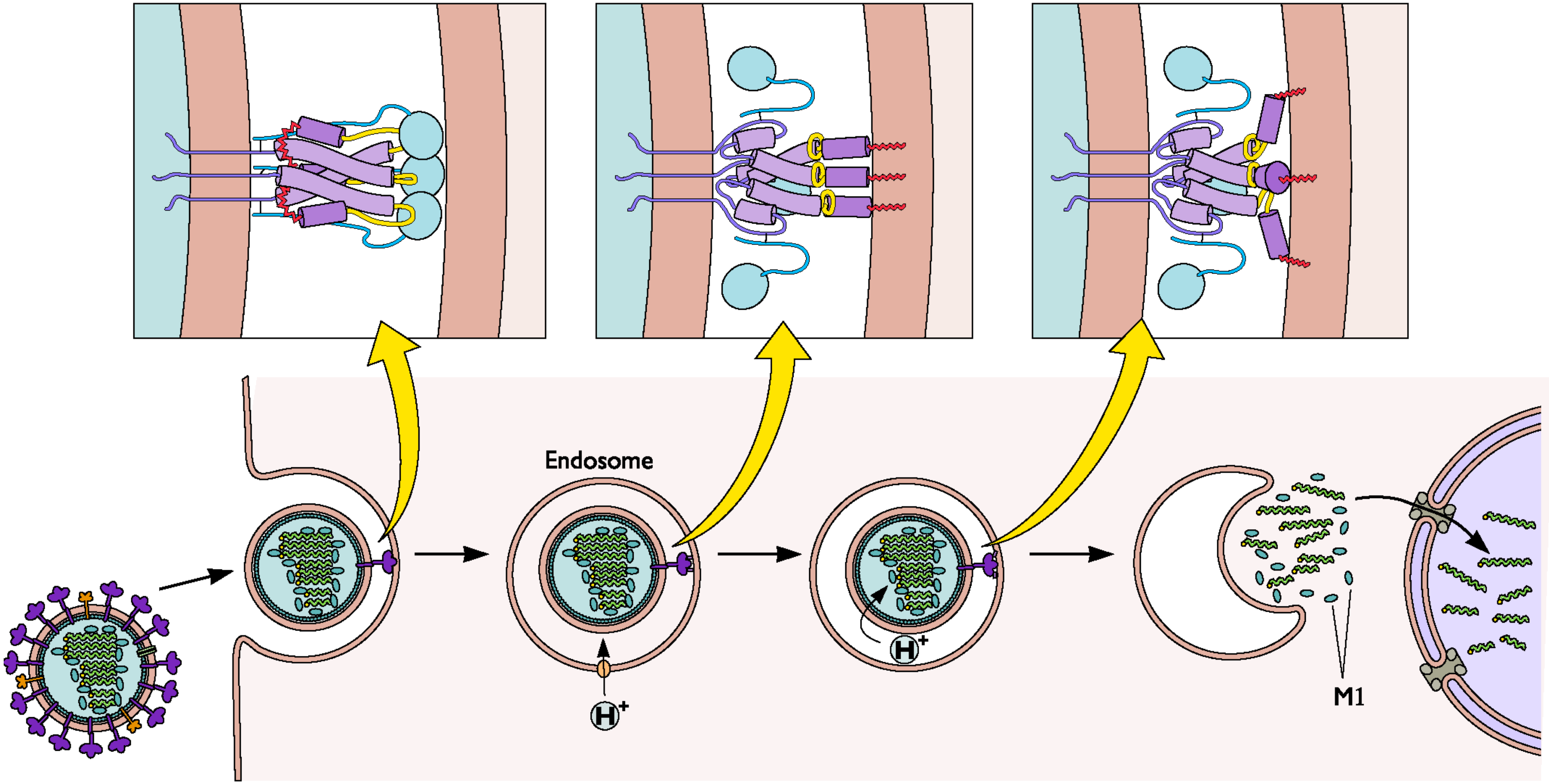




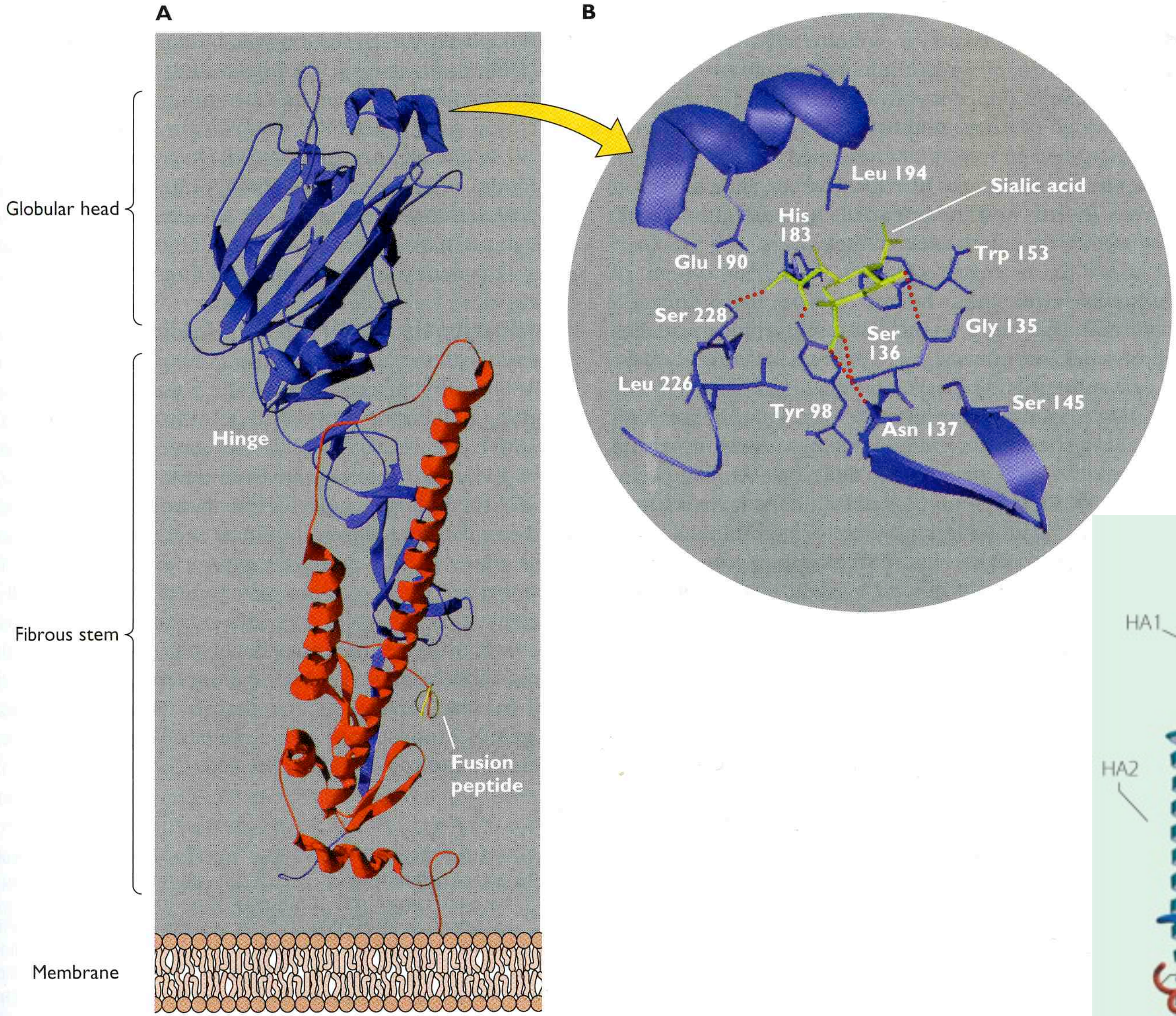
Virus entry via receptor-mediated endocytosis

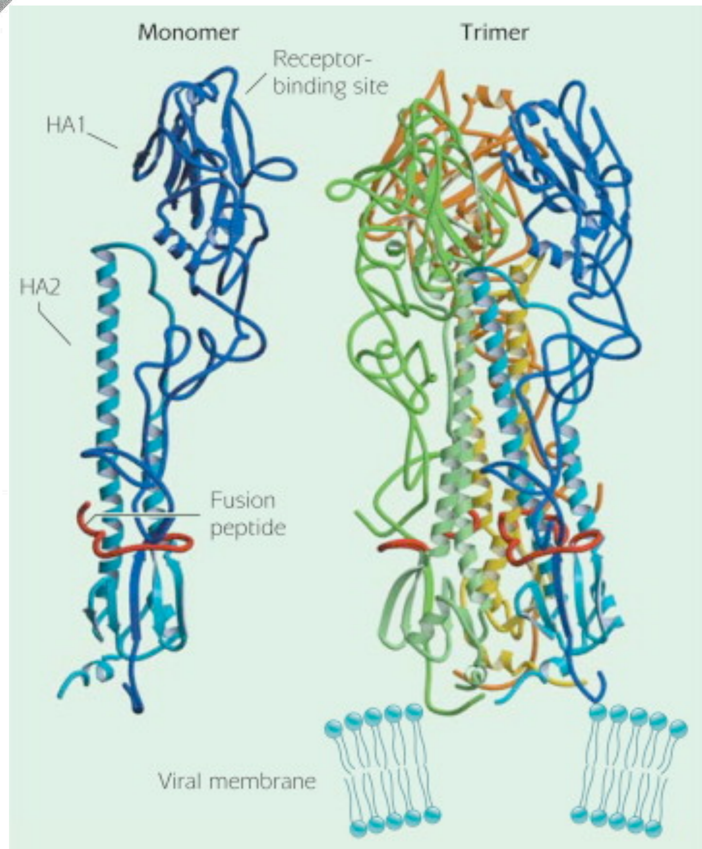


Influenza virus: an example of virus entry via the clathrin-dependent receptor-mediated endocytic pathway

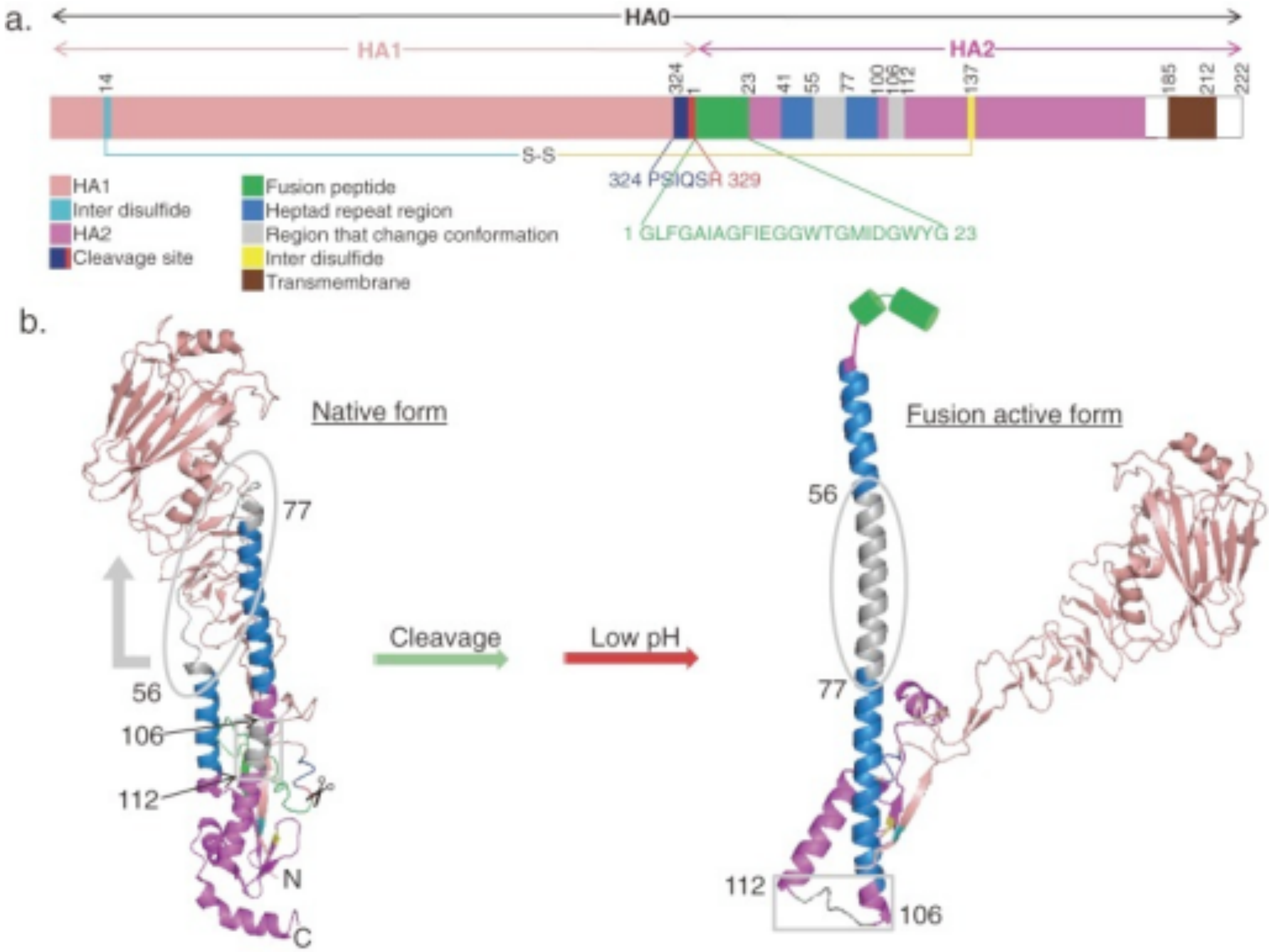


Structure of HA

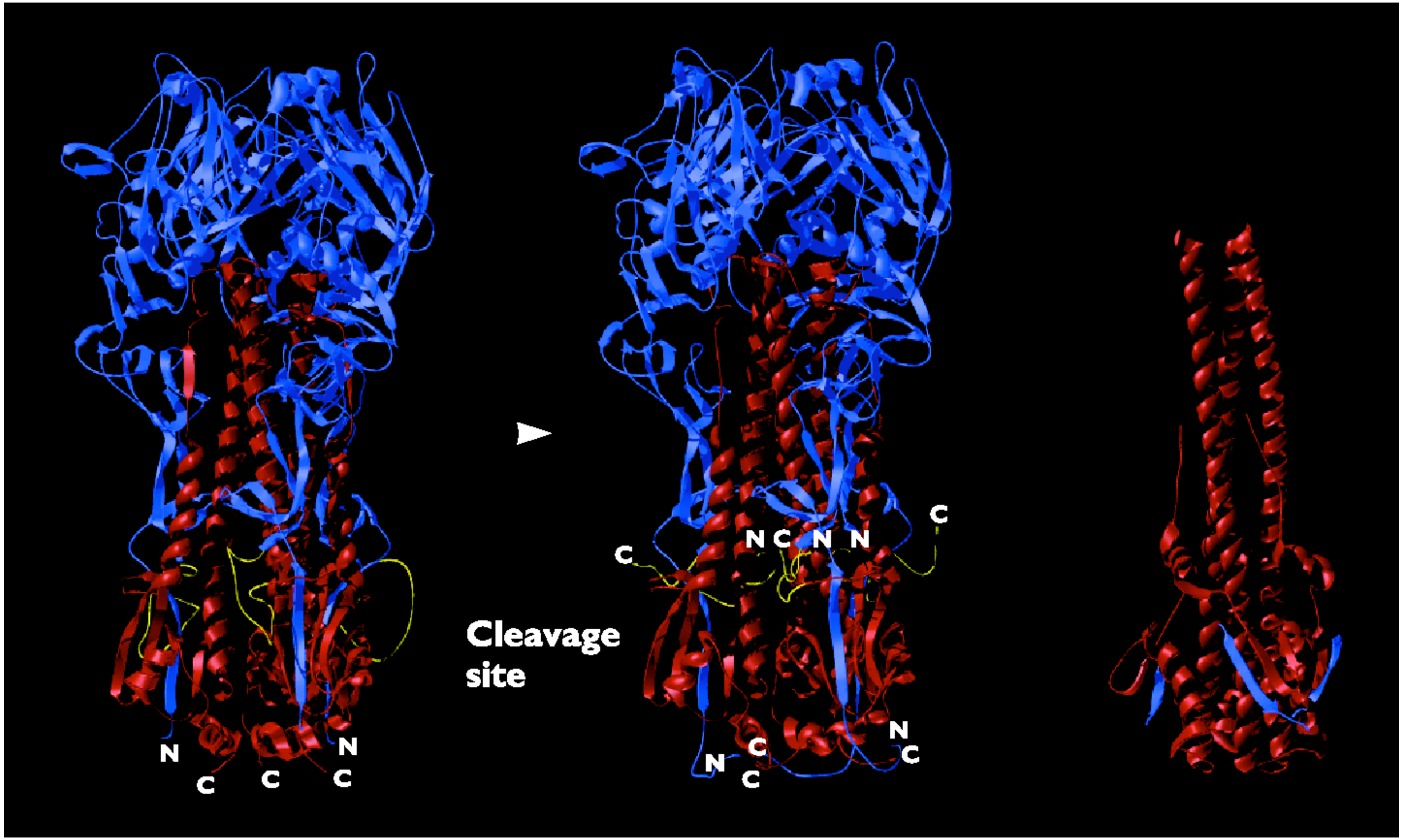




Conformational changes of HA at the pH of membrane fusion



Cleavage- and low-pH-induced structural changes in the Influenza virus HA

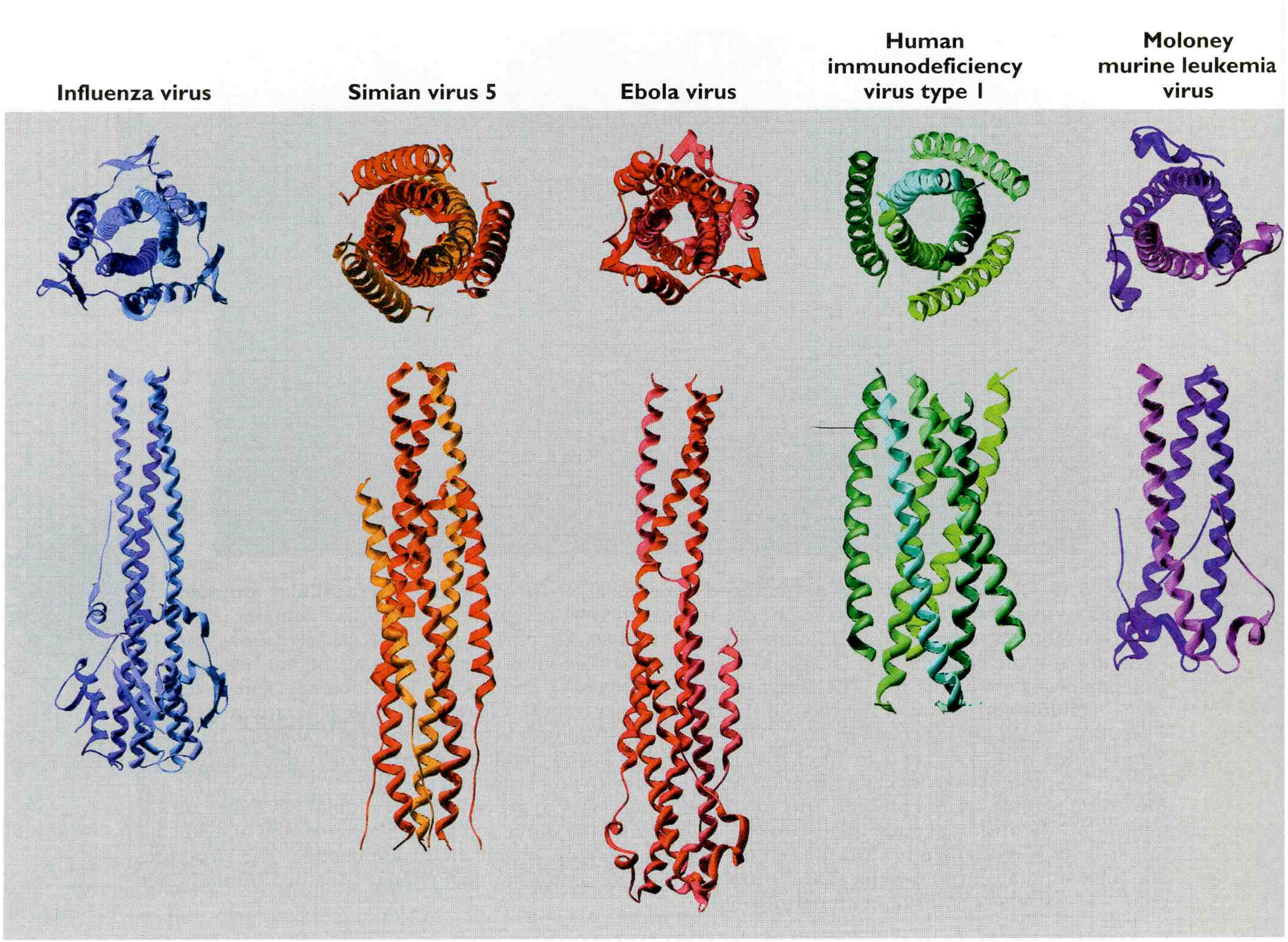


Uncleaved HAO precursor

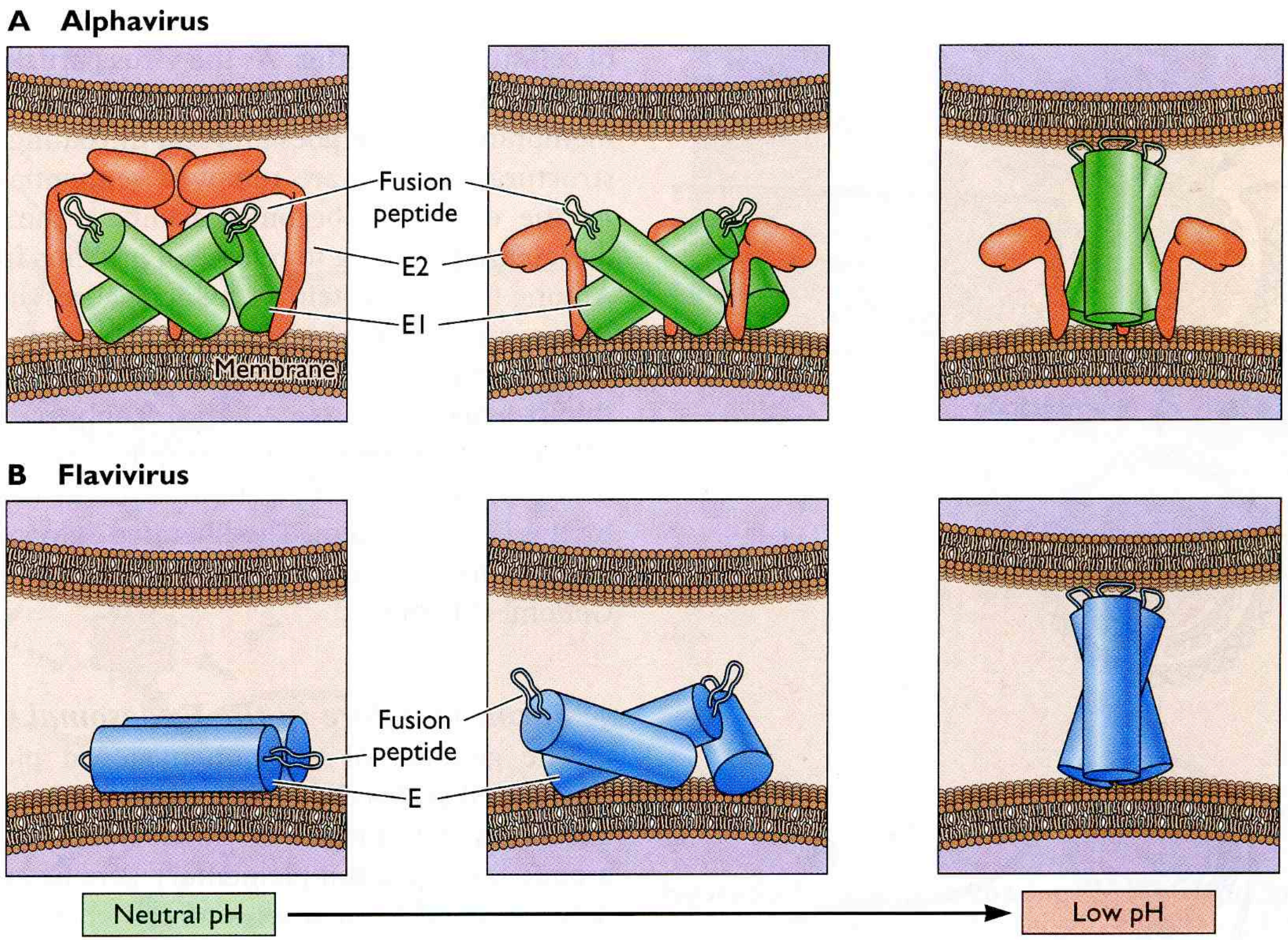
Structure of the HA trimer at neutral pH

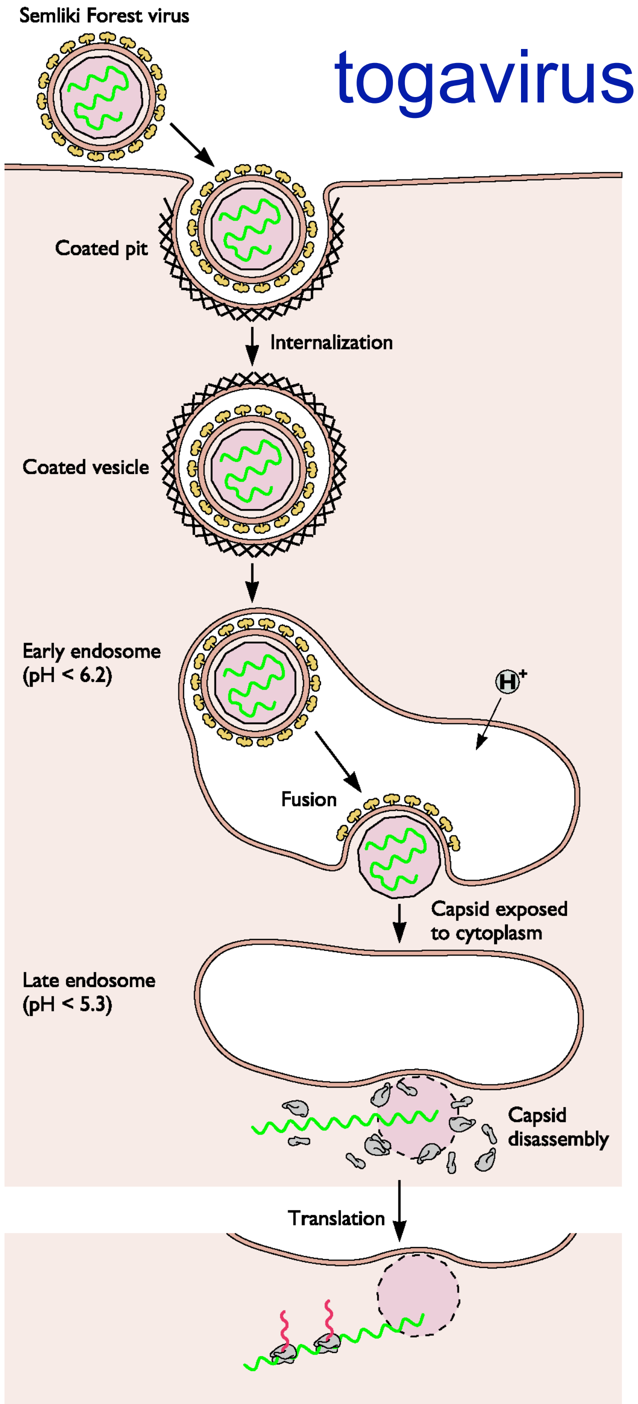
Structure of the low pH trimer (only HA2 is shown)

Similarities among five viral fusion protein

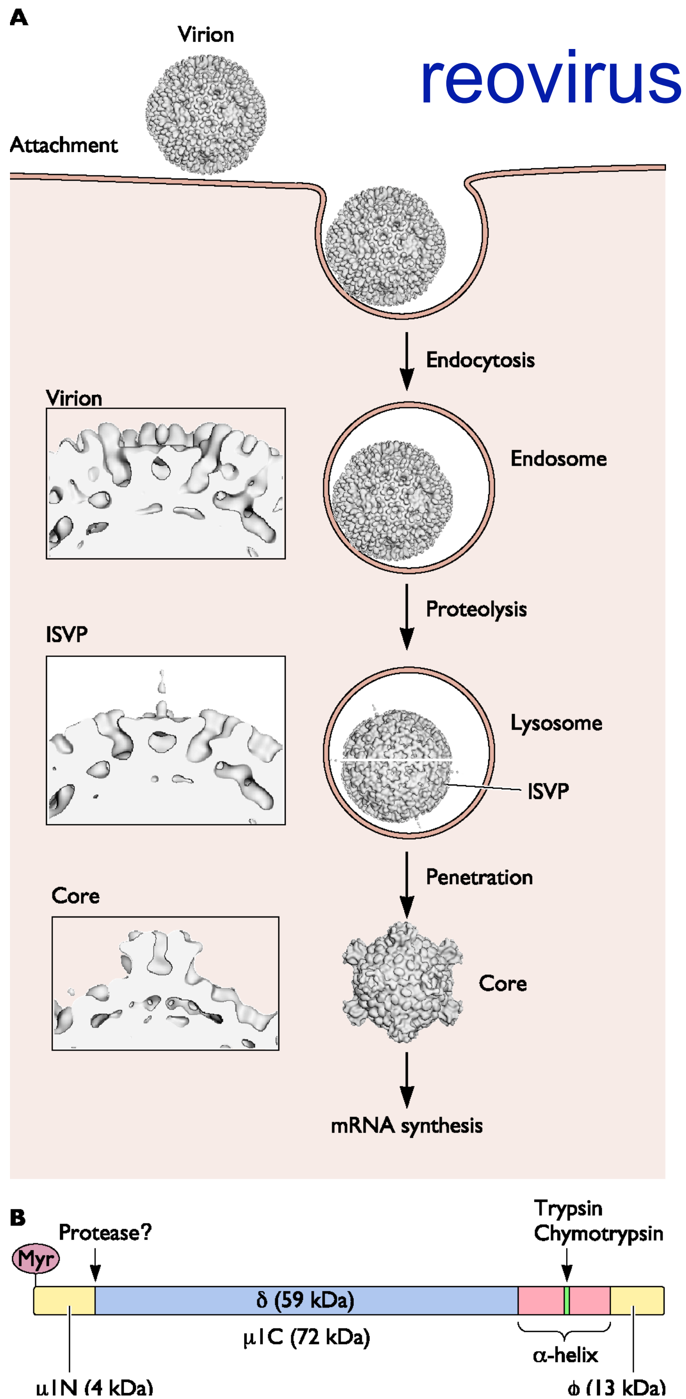


Model for low-pH-induced movement of alphavirus and flavivirus glycoproteins

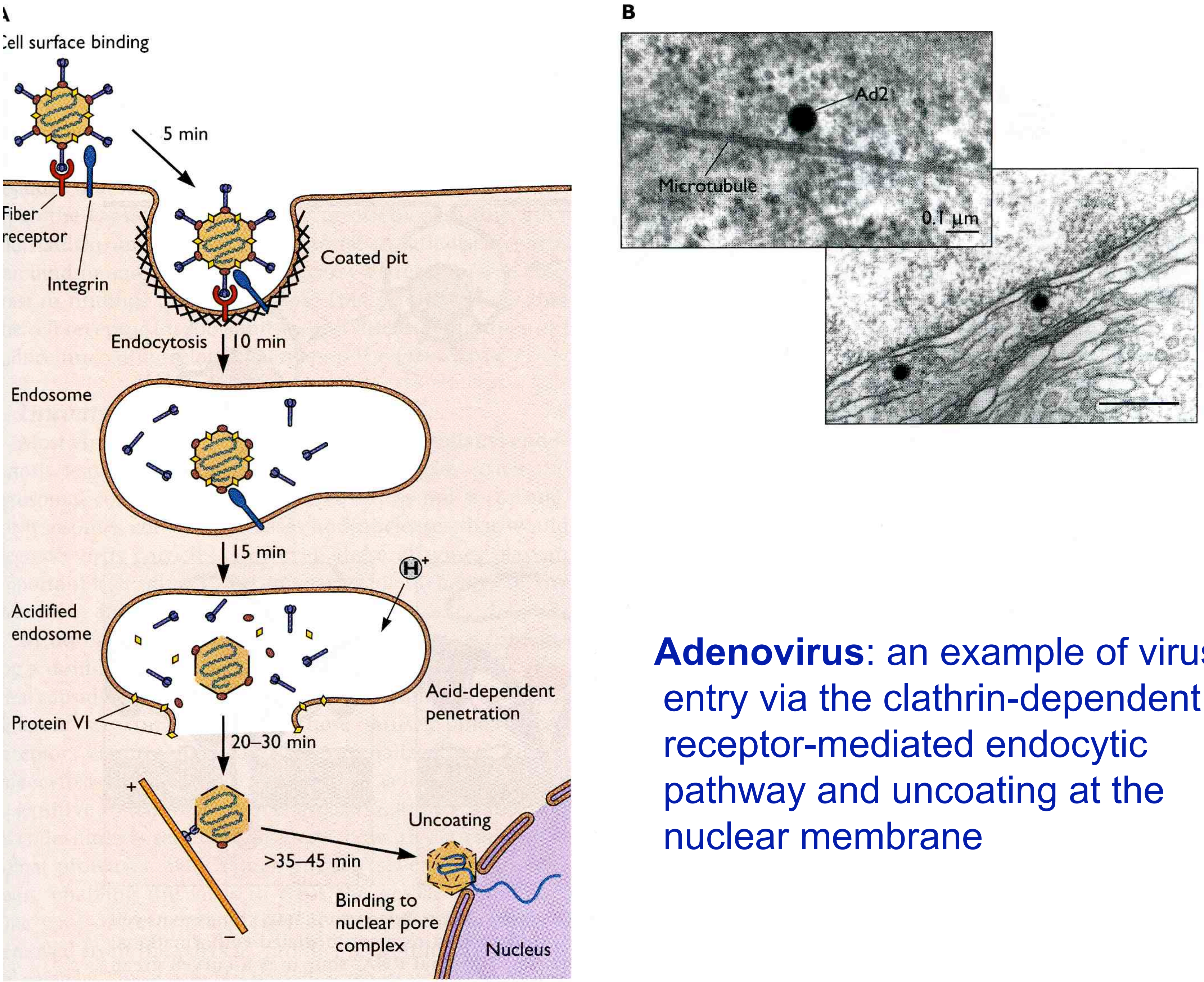




togavirus
Semliki Forest virus
Coated pit
Internalization
Coated vesicle
Early endosome
(pH < 6.2)
H+
Fusion
Capsid exposed
to cytoplasm
Late endosome
(pH < 5.3)
Capsid
disassembly
Translation

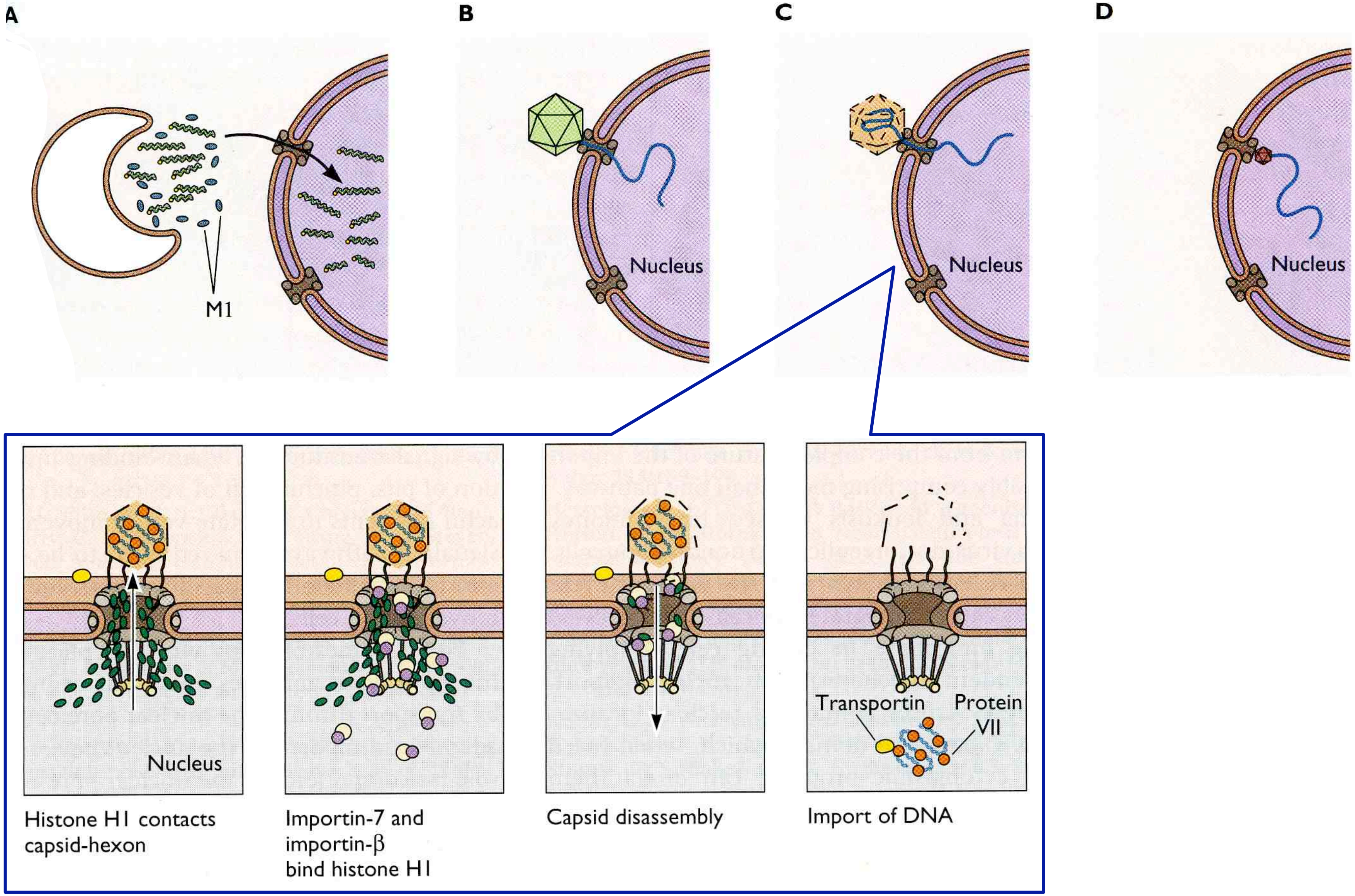


A
reovirus
Virion
Attachment
Endocytosis
Virion
Endosome
Proteolysis
ISVP
Lysosome
ISVP
Penetration
Core
Core
mRNA synthesis
B
Myr
Protease?
Trypsin
Chymotrypsin
δ (59 kDa)
μ1C (72 kDa)
α-helix
μ1N (4 kDa)
ϕ (13 kDa)

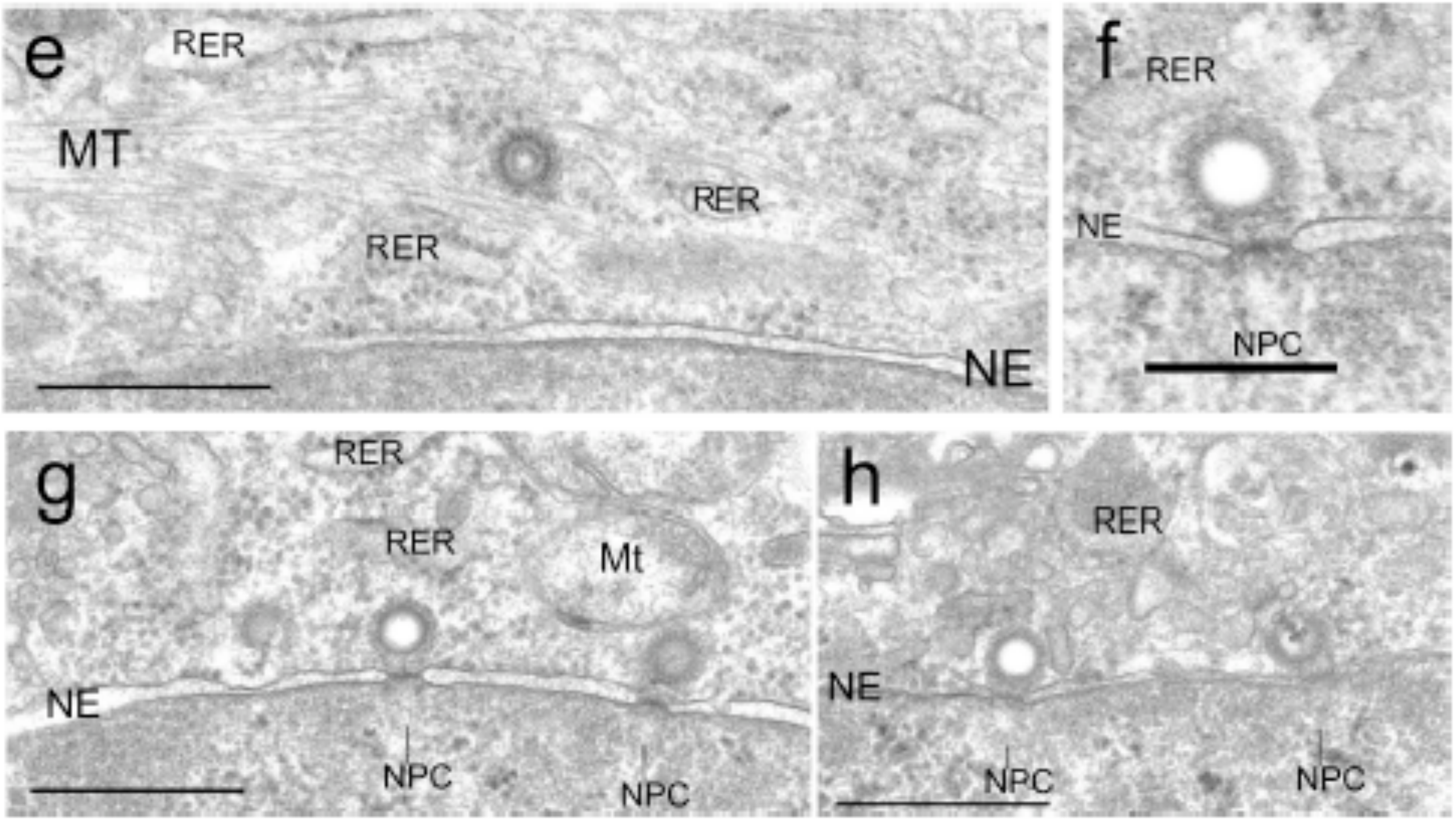


Adenovirus: an example of virus entry via the clathrin-dependent receptor-mediated endocytic pathway and uncoating at the nuclear membrane

Different strategies for entering the nucleus



The microtubule network mediates nuclear targeting of Human Cytomegalovirus capsids



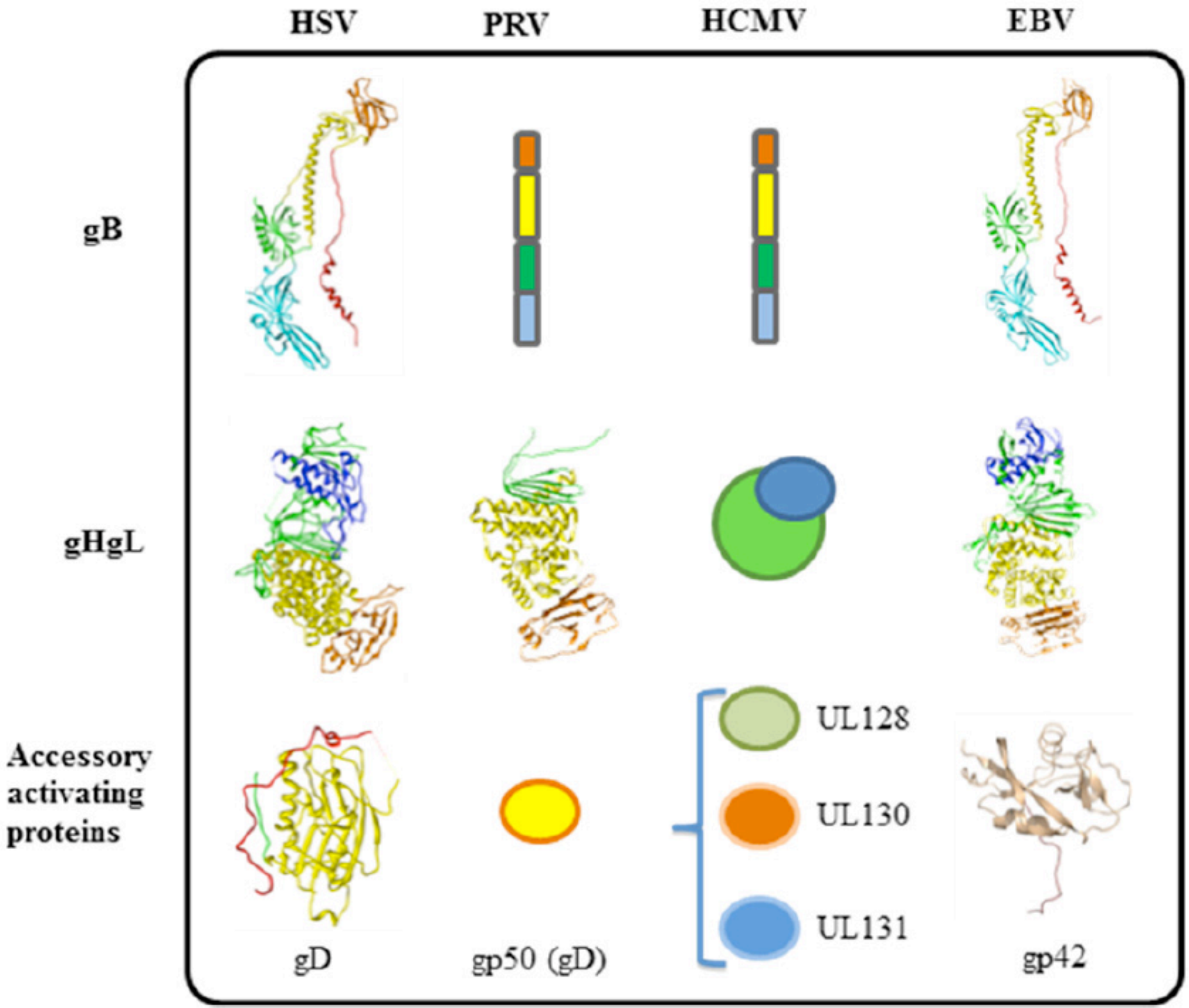
(Ogawa-Goto et al., 2003)

The infectious cycle

virus entry into host cells:

Herpesvirus entry

Fusion machinery of Herpesviruses



Eisenberg et al., 2012

The basic steps in Herpes simplex virus entry

Herpes simplex virus enter cells via direct fusion with the plasma membrane at neutral pH, or by endocytosis.

The fusion is a process that consists of three basic steps carried out by the virion glycoproteins:

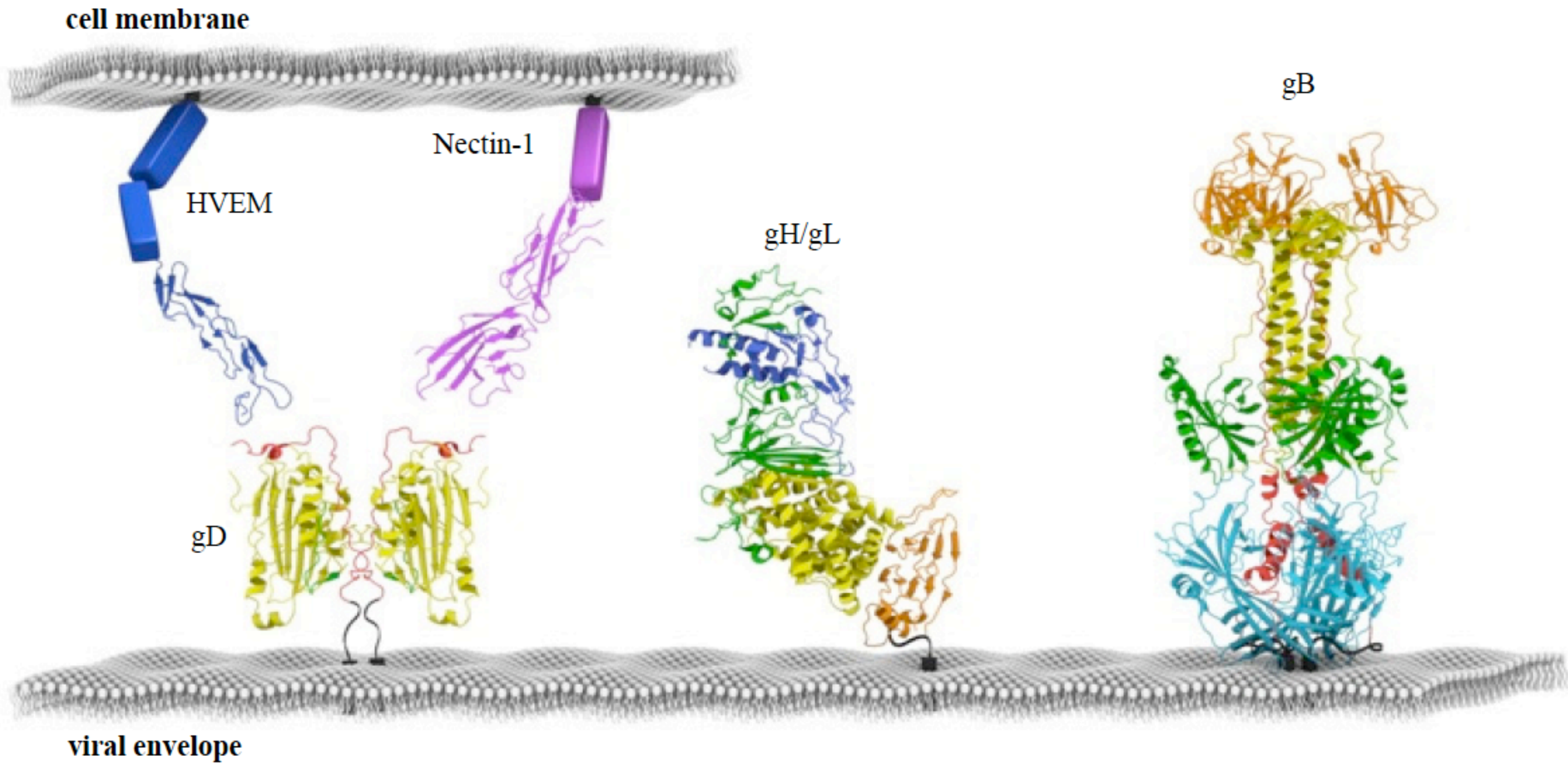
1) **recognition of a cellular receptor by a viral glycoprotein**

2) **triggering of fusion**

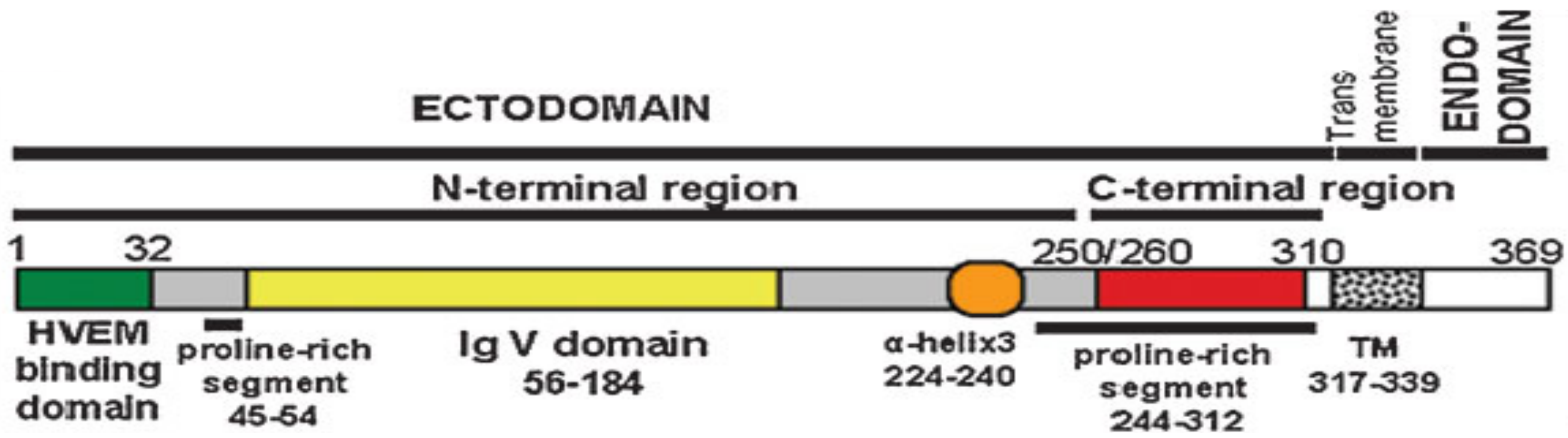
3) **fusion execution**

These steps are carried out by four essential virion glycoproteins: **gB, gD, gH** and **gL.**

Fusion machinery of HSV-1



Structure and function of HSV **gD** glycoprotein



Campadelli *et al.*, 2007

- **369 aa**
- **organized in three domains:**

  1) ecto-domain: aa 1-317

     N-terminal: aa 1-260
     core: aa 56-184;
     α-helix, aa 224-240
     C-terminal: aa 261-310

  2) trans-membrane domain: aa 318-339
  3) endo-domain: aa 340-369

gD functions:

1) **receptor recognition**

2) **triggering of fusion**

Functions of HSV **gD**: receptor recognition

The three natural gD receptors:

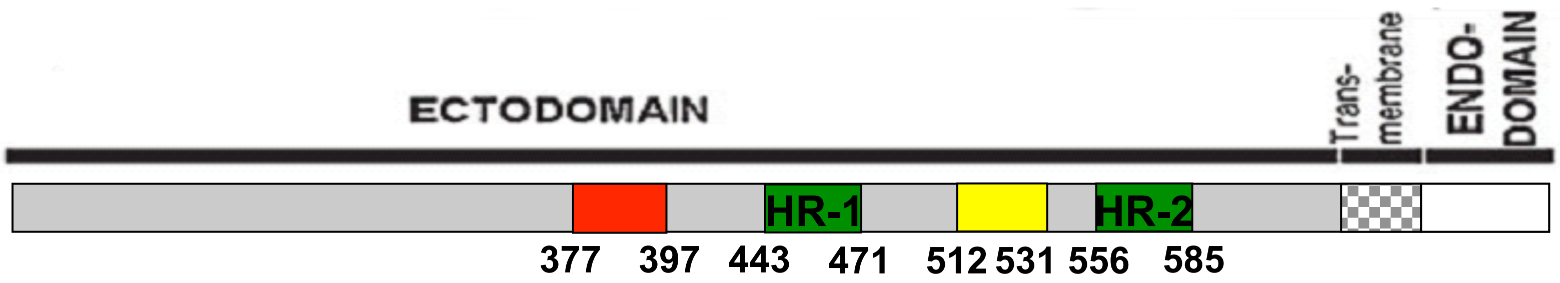
1. **herpesvirus entry mediator (HVME):**
   tumor necrosis factor receptor family;
   in T-lymphocytes or lymphoyd organ.
   HVEM binding-site: aa 1-32 (contact residues between aa 7-15 and 24-32).

2. **nectin 1:**
   intercellular adhesion molecules family;
   in sensory neurons, muco-epithelia or epithelia cells.
   nectin-1 binding-site: critical aa residues (aa 34, 38, 215 and aa 222-223).

3. **O-sulphated HS (heparan sulfate):**
   modified heparan sulfate by enzymes in neuronal and endothelial
   cells, corneal fibroblasts.

Structure and function of HSV **gH**

HSV-1 gH exibits structural and functional features typical of class I viral fusion gp



- **838 aa**

- **organized in three domains:**

1) ecto-domain
   heptad repeats: HR-1 (aa 443-471)
   HR-2 (aa 556-585);

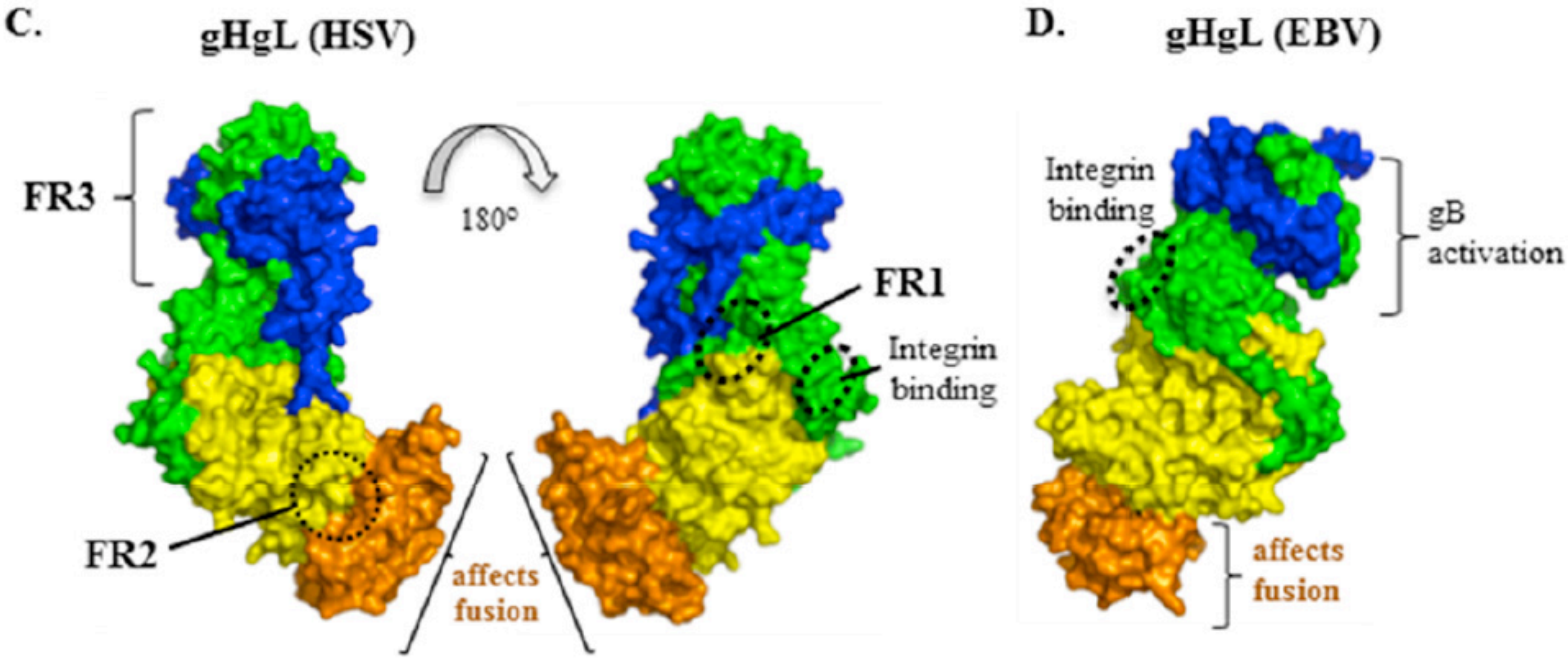
2) trans-membrane domain
3) endo-domain

gH functions:

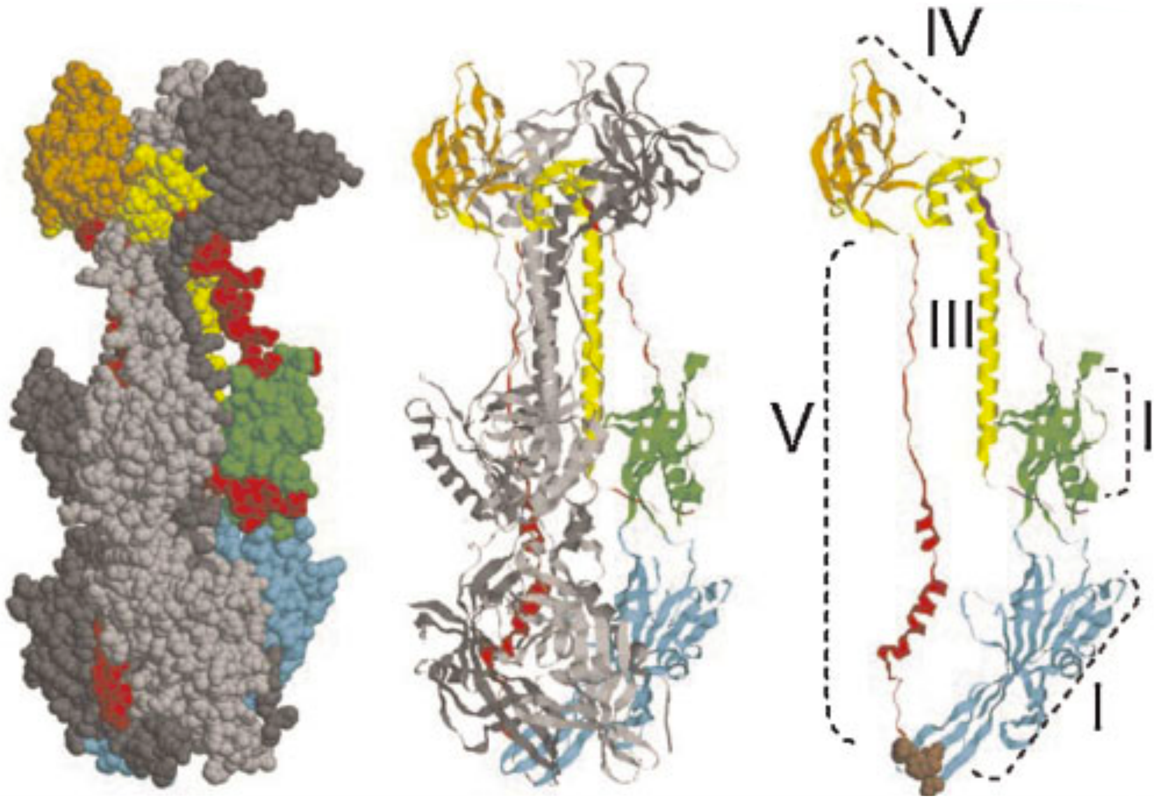
1) interactions with gD and gB

2) triggering of fusion

Structure and function of HSV **gH**



Structure and function of HSV **gB**: an effector of membrane fusion



Campadelli *et al.*, 2007

- **904 aa**
- **trimer with a coiled-coil core**
- **organized in three domains:**
  1) ecto-domain: 696 aa
  α-helix III,
  HR-1 (aa 92-112)
  HR-2 (618-631)

  2) trans-membrane domain:69 aa

  3) endo-domain: 109 aa

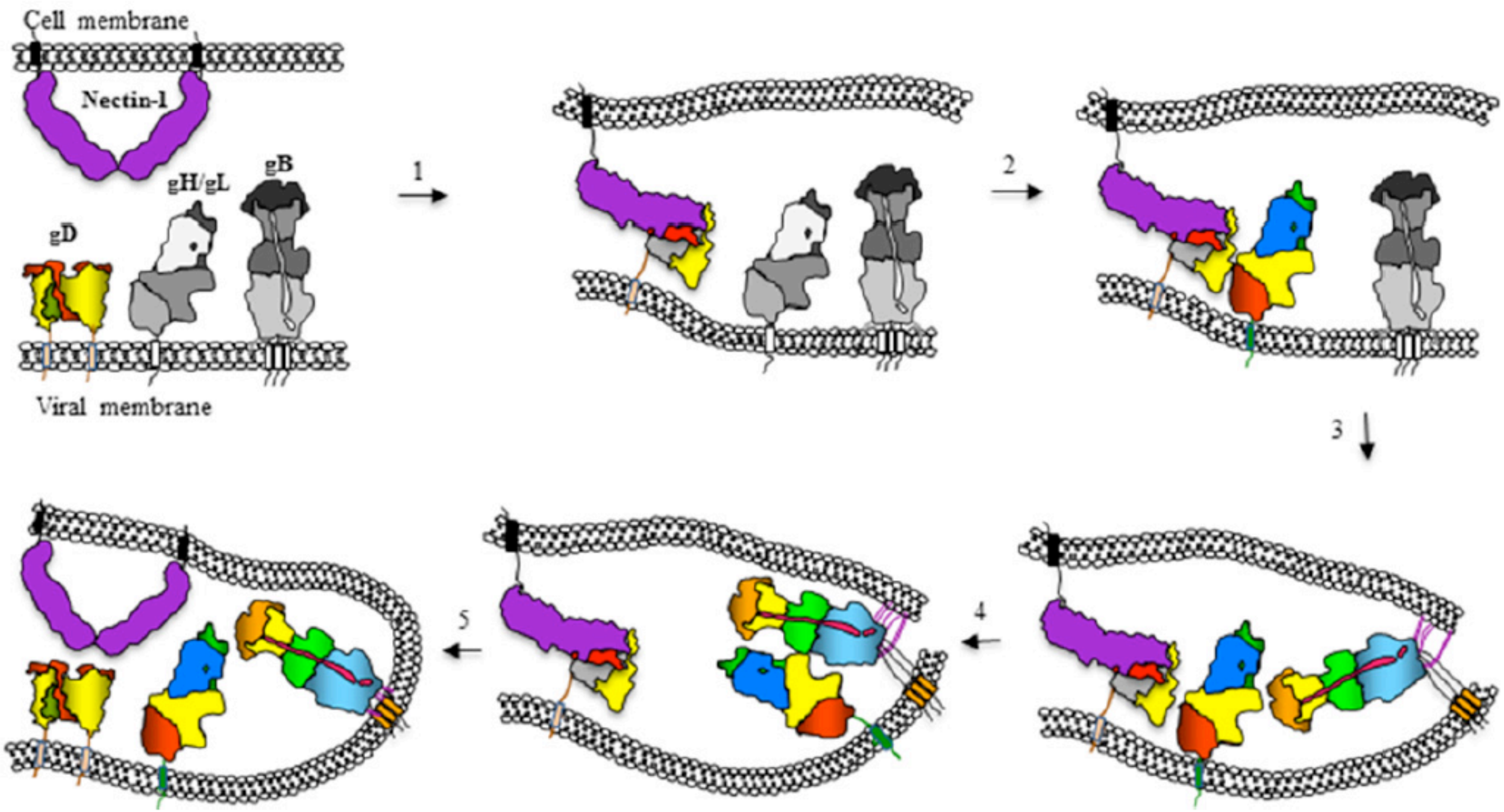
gH functions:

1) Binding to HSPG

2) Fusion execution

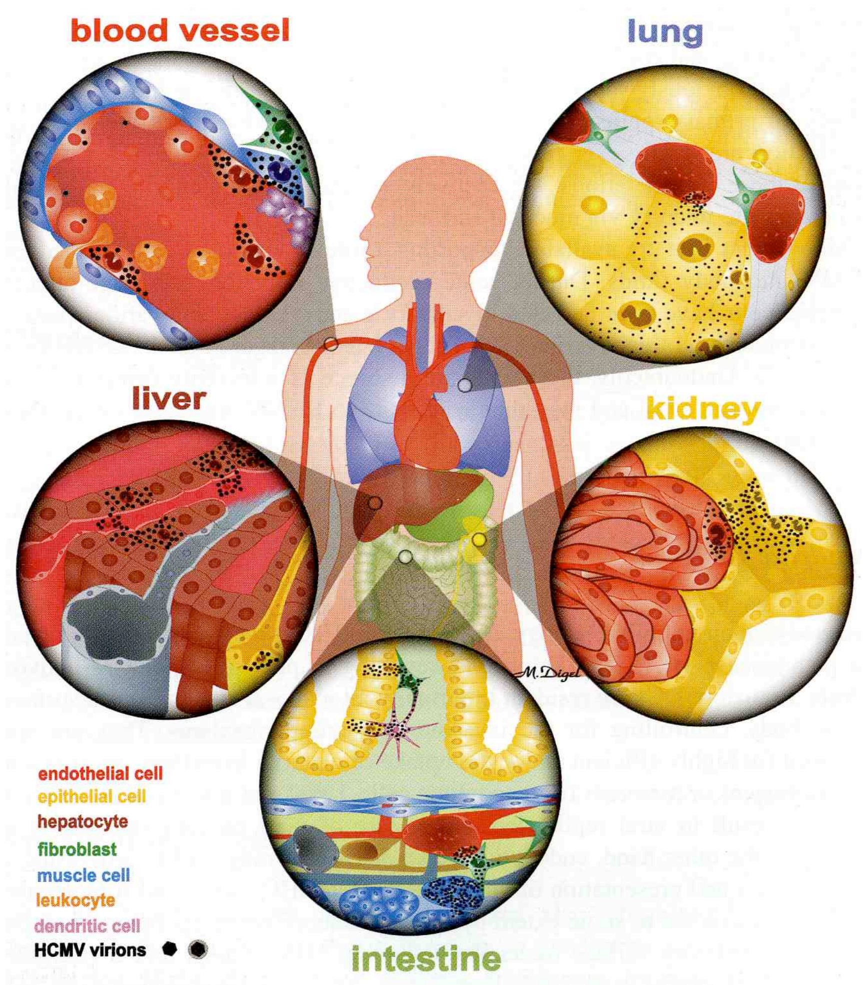
HSV-1 gH exibits structural and functional features typical of Class III viral fusion gp

Working model for HSV entry into cells

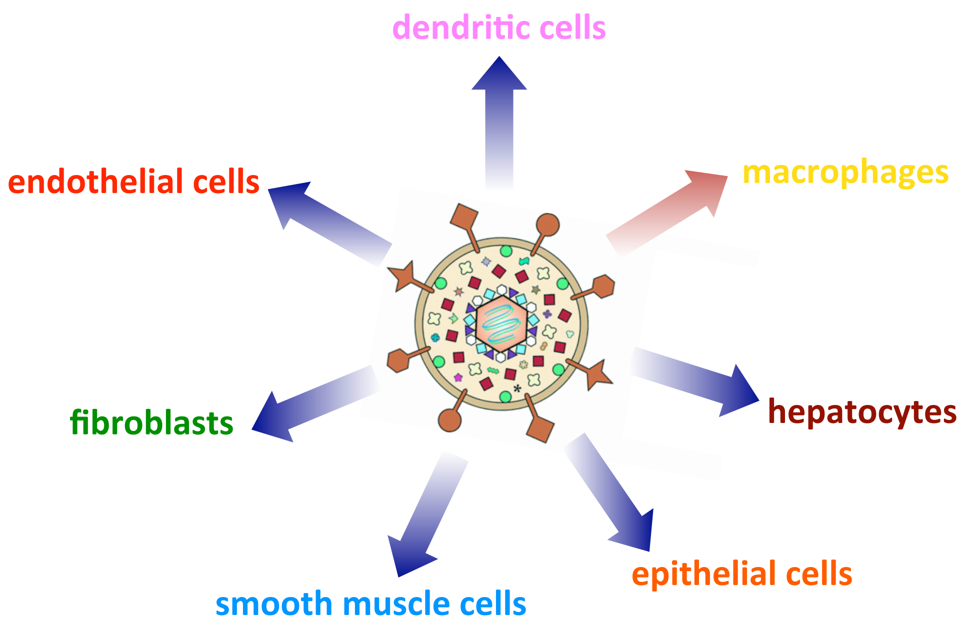


Eisenberg et al., 2012

The broad cell tropism of HCMV

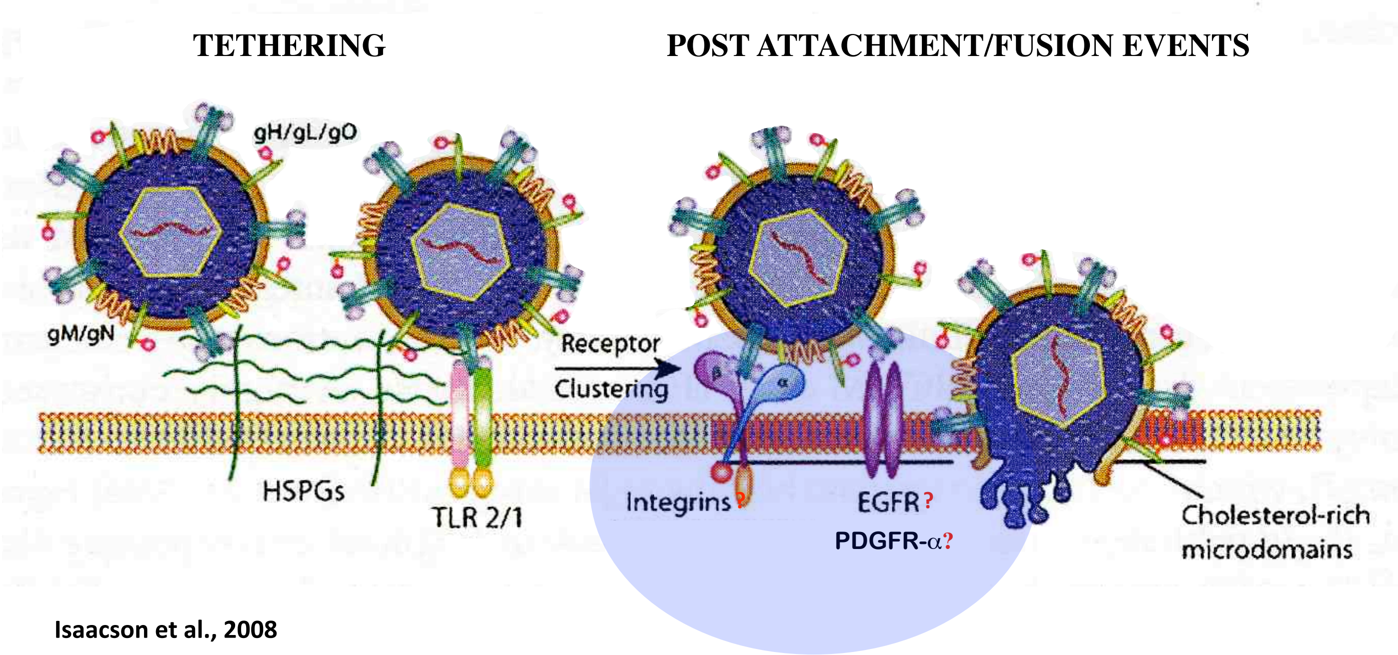


Sinzger et al., 2008



The broad spectrum of cell types infected *in vivo* greatly contributes to the pathogenesis of HCMV diseases

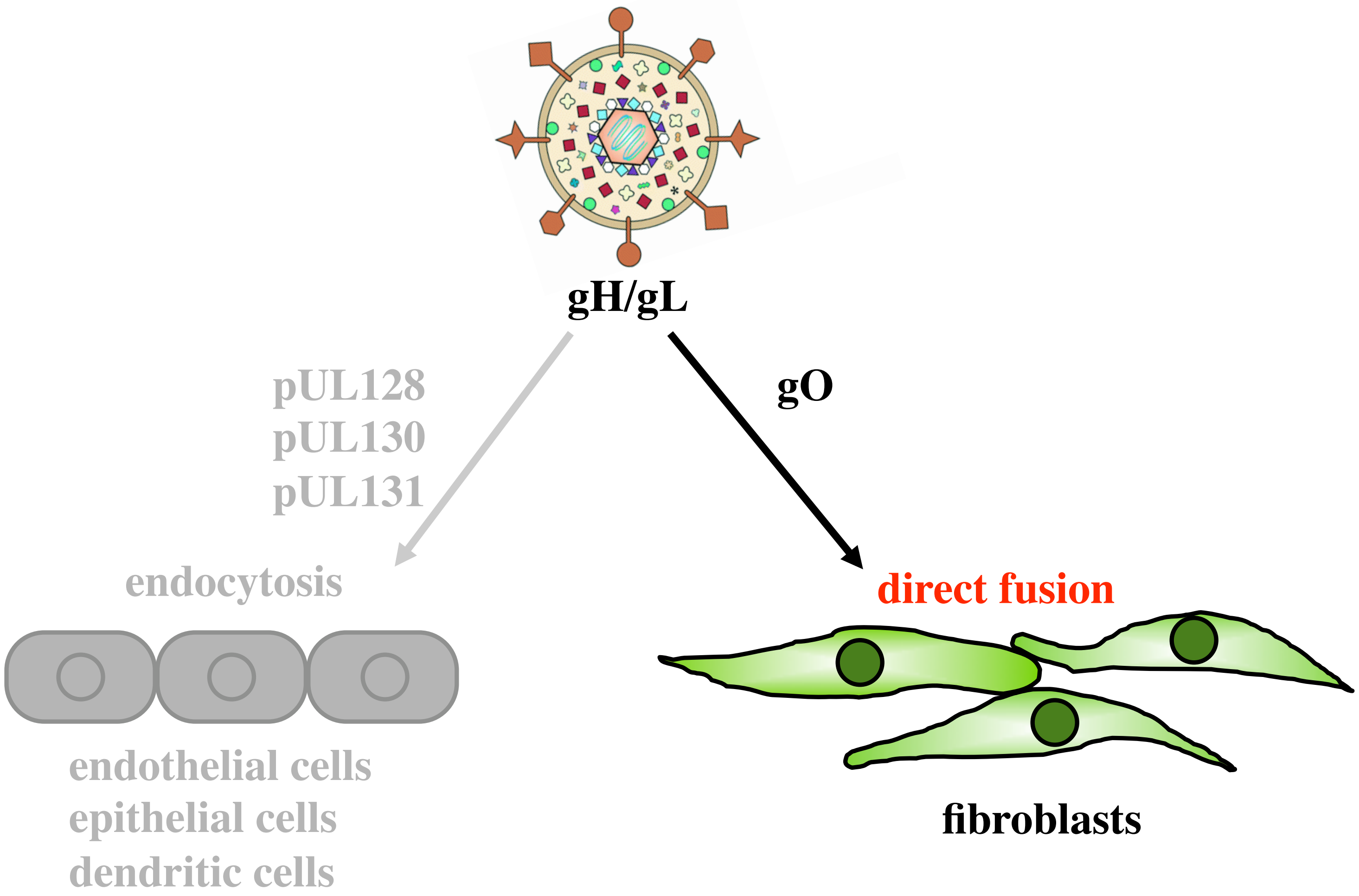
The broad cell tropism of HCMV: different receptor utilization



Isaacson et al., 2008

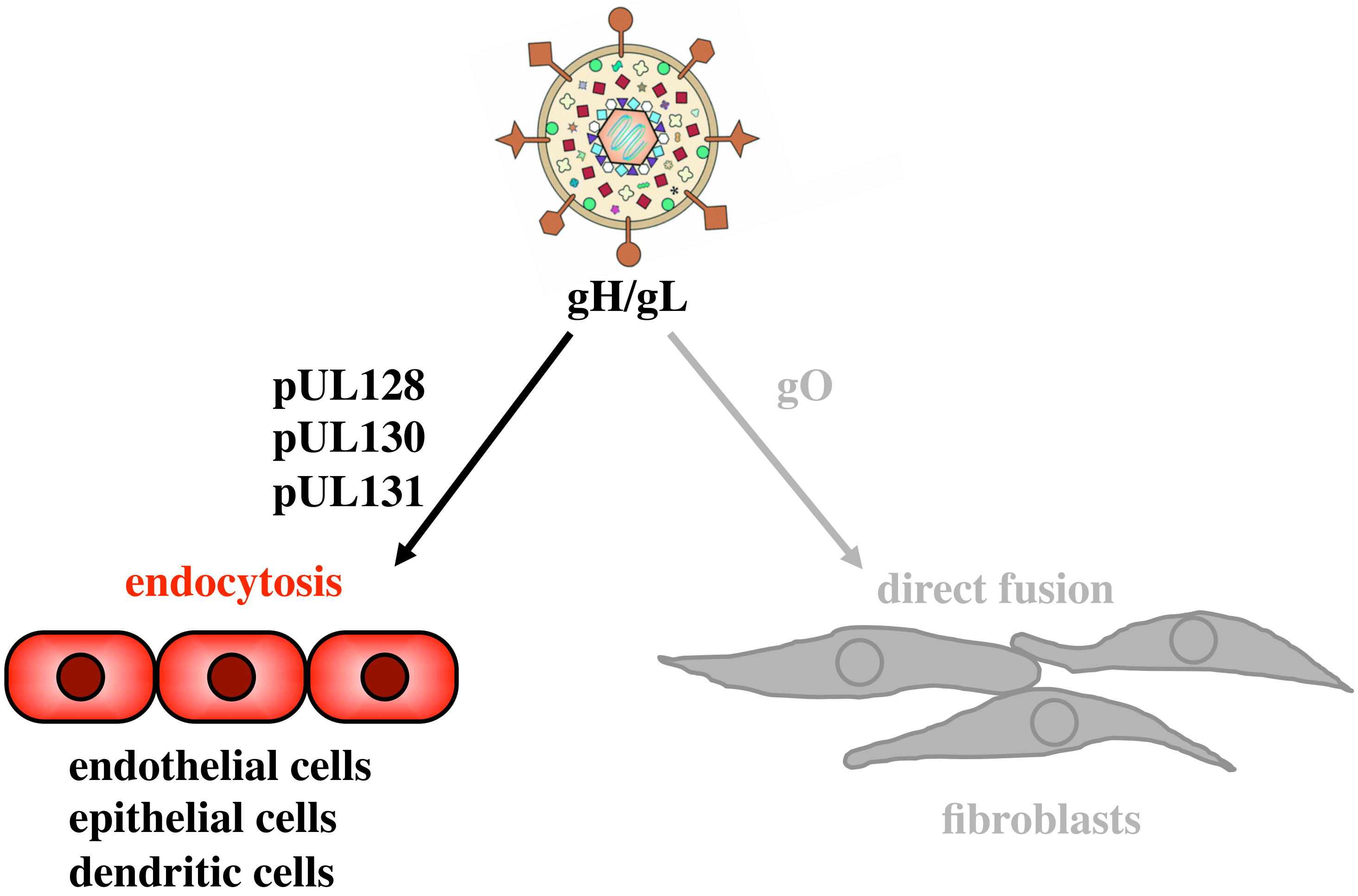
Presence of multiple and/or ubiquitously expressed cellular receptors

The broad cell tropism of HCMV: different envelope glycoproteins and tropism factors requirements



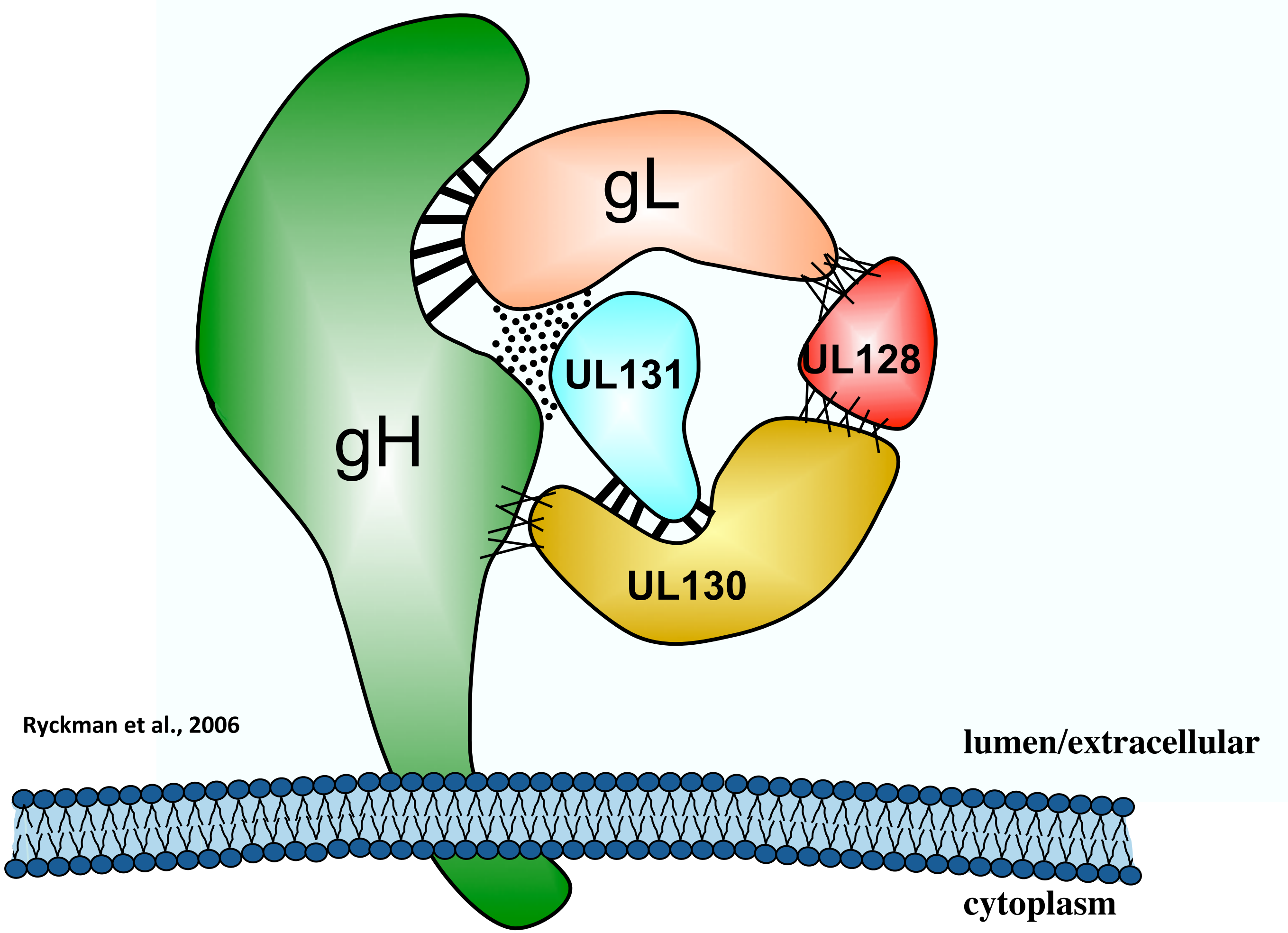
Presence of modular envelope complexes that mediate viral entry in different cell types by different pathways

The broad cell tropism of HCMV: different envelope glycoproteins and tropism factors requirements



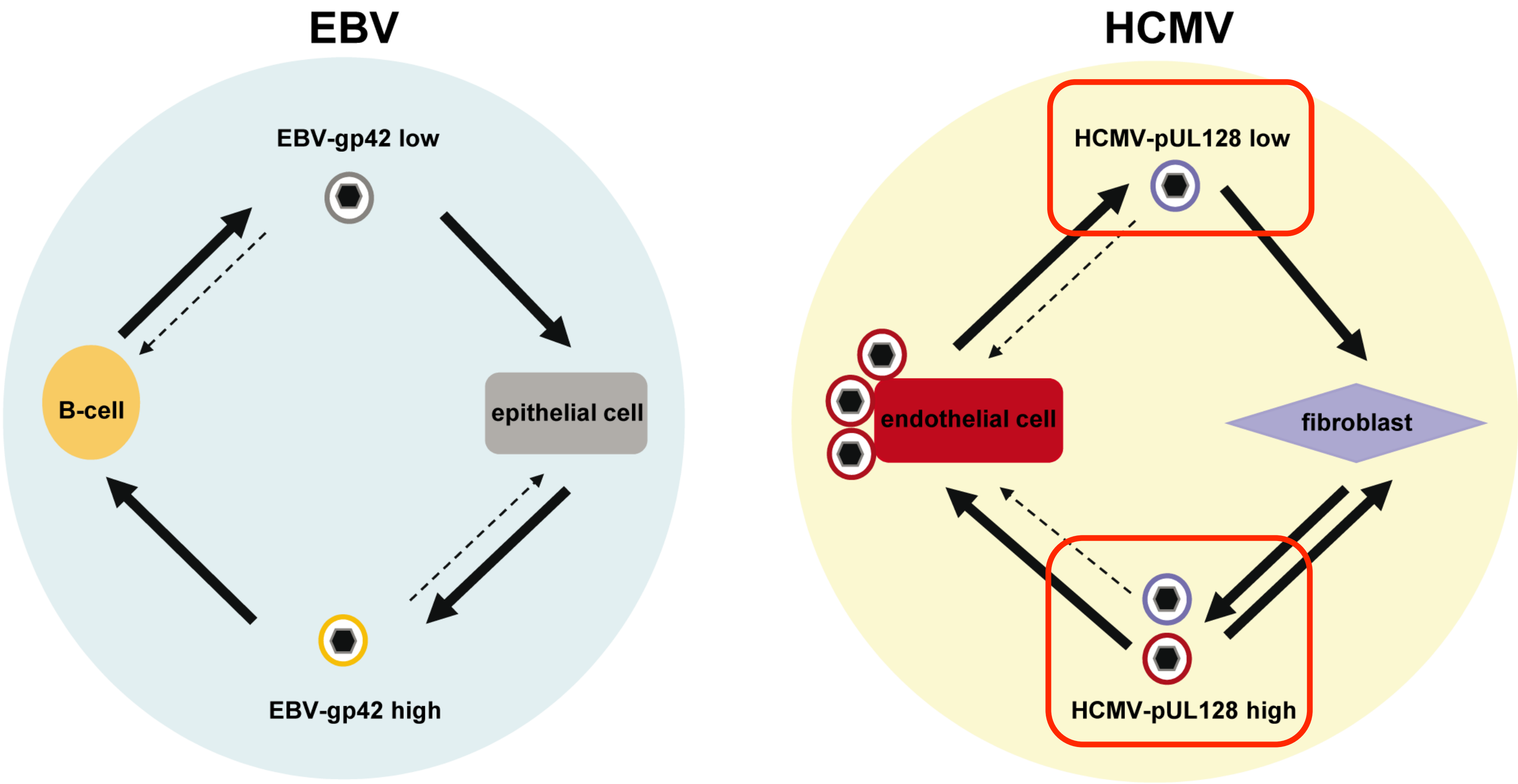
Presence of modular envelope complexes that mediate viral entry in different cell types

The broad cell tropism of HCMV: the role of pUL tropism factors



pUL (UL128, UL130 and UL131) proteins assemble onto a gH/gL scaffold to form a virion complex that mediates entry in epithelial and endothelial cells.

The presence of different Herpesvirus envelope complexes may switch route of infection in vivo



switch of cell tropism

switch of spread mode

Scrivano et al., 2011