

# RNA VIRUS REPLICATION STRATEGIES

(excluding hepatitis delta “virus”/“agent” replication strategy)

# RNA VIRUS STRATEGIES

## RNA -> RNA

RNA-dependent RNA polymerase

## RNA -> DNA

RNA-dependent DNA polymerase  
- reverse transcriptase

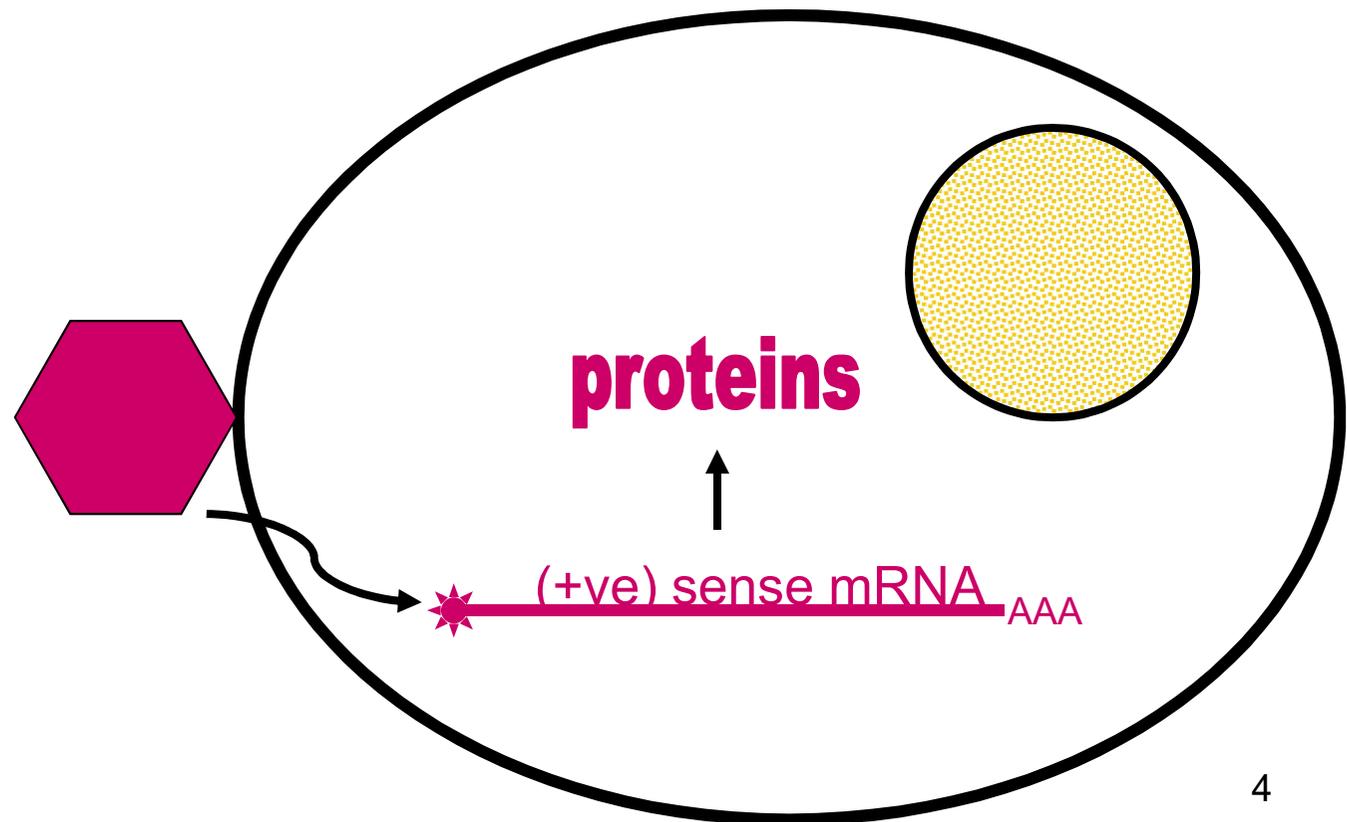
## Host cell DNA -> RNA

DNA-dependent RNA polymerase

ALL ANIMAL RNA VIRUSES  
CODE FOR A POLYMERASE

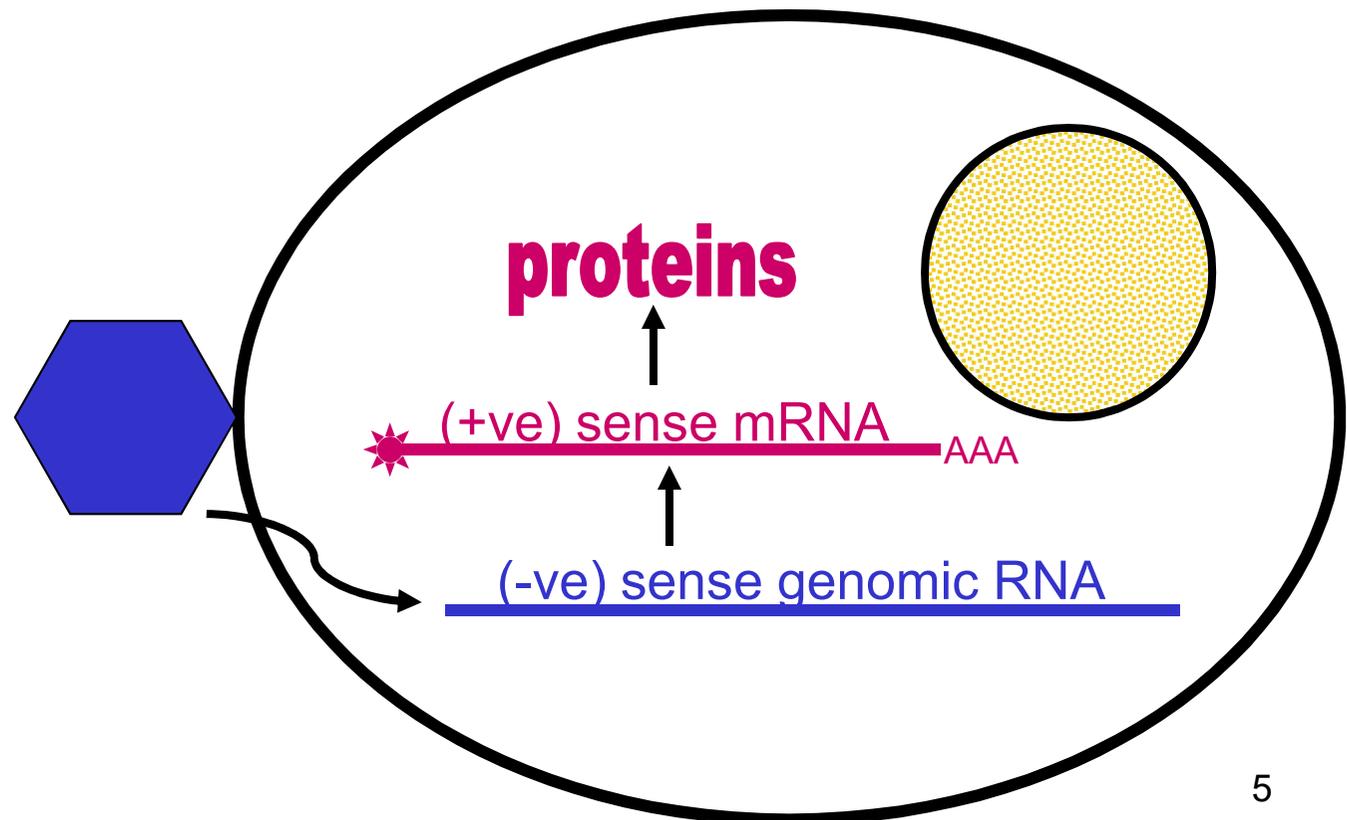
# Need to make mRNA

PLUS (POSITIVE) SENSE RNA GENOMES



# Need to make mRNA

MINUS (NEGATIVE) SENSE RNA GENOMES

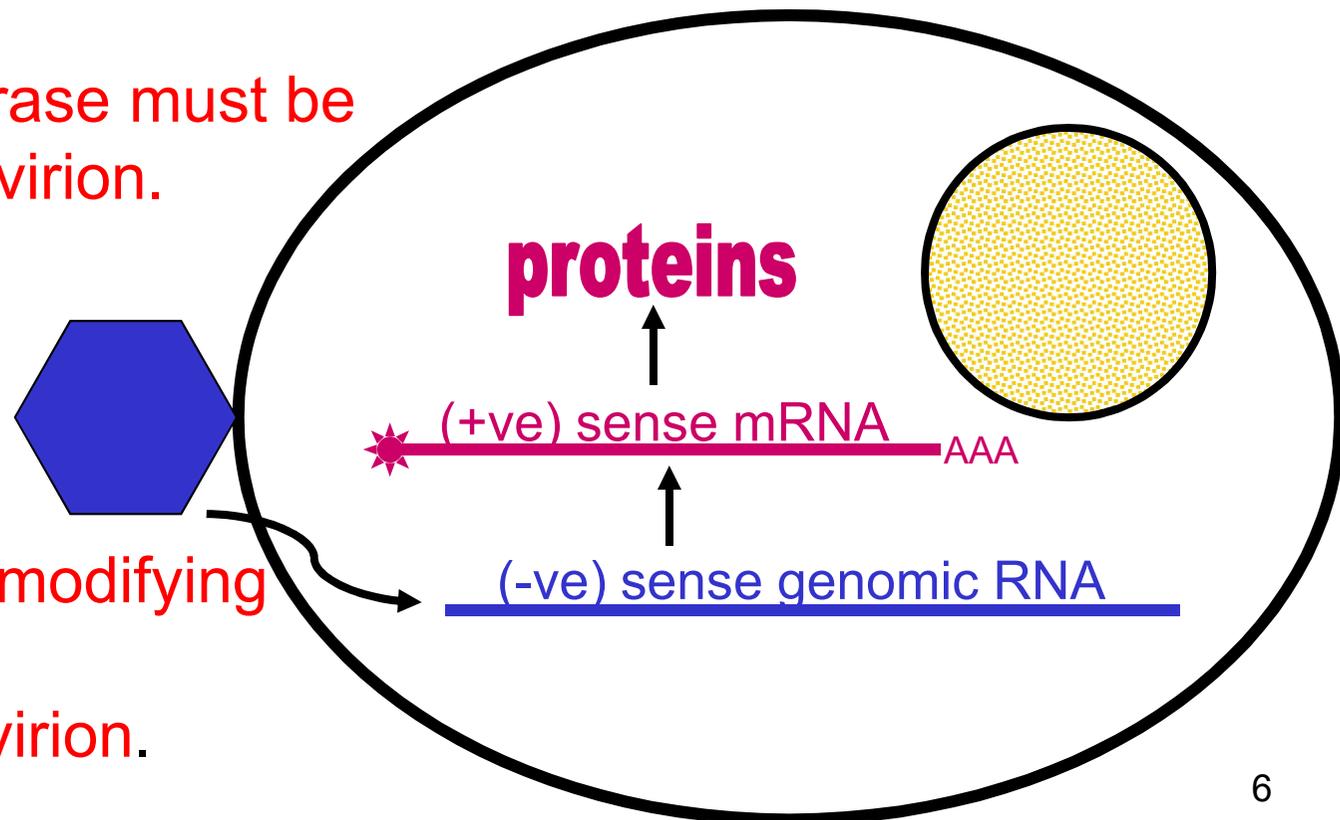


# Need to make mRNA

## MINUS (NEGATIVE) SENSE RNA GENOMES

RNA polymerase must be packaged in virion.

If used, RNA modifying enzymes are packaged in virion.

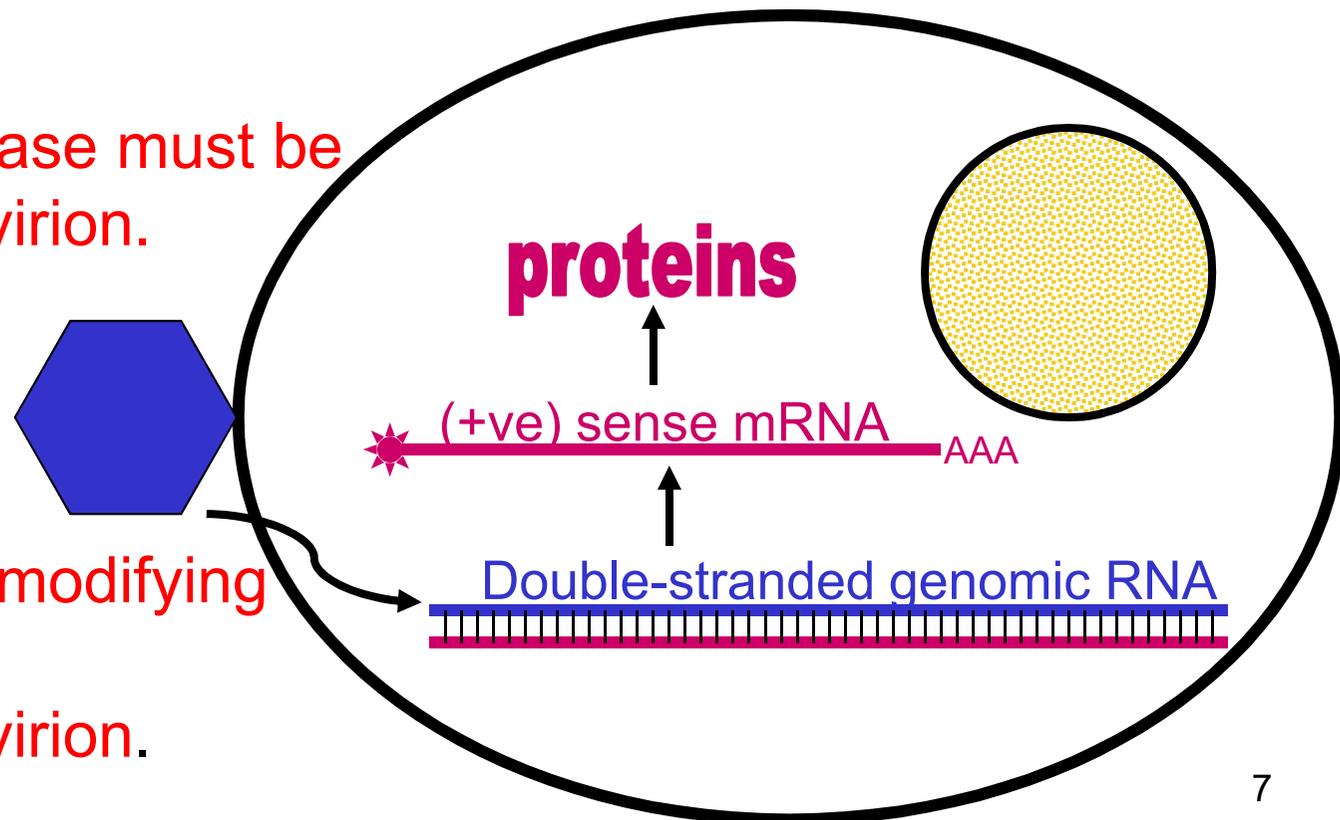


# Need to make mRNA

## DOUBLE-STRANDED RNA GENOMES

RNA polymerase must be packaged in virion.

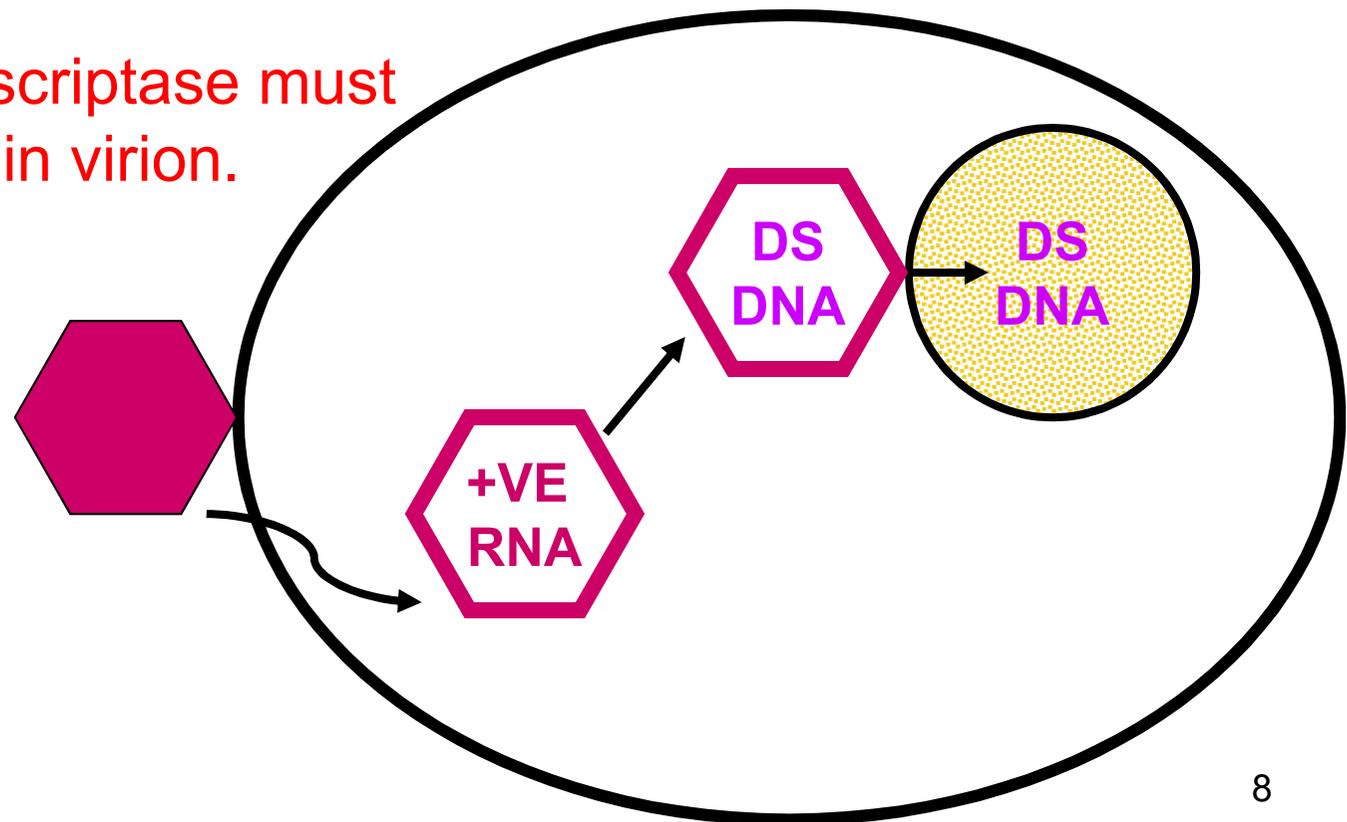
If used, RNA modifying enzymes are packaged in virion.



# Need to make mRNA

## RETROVIRUSES

Reverse transcriptase must be packaged in virion.



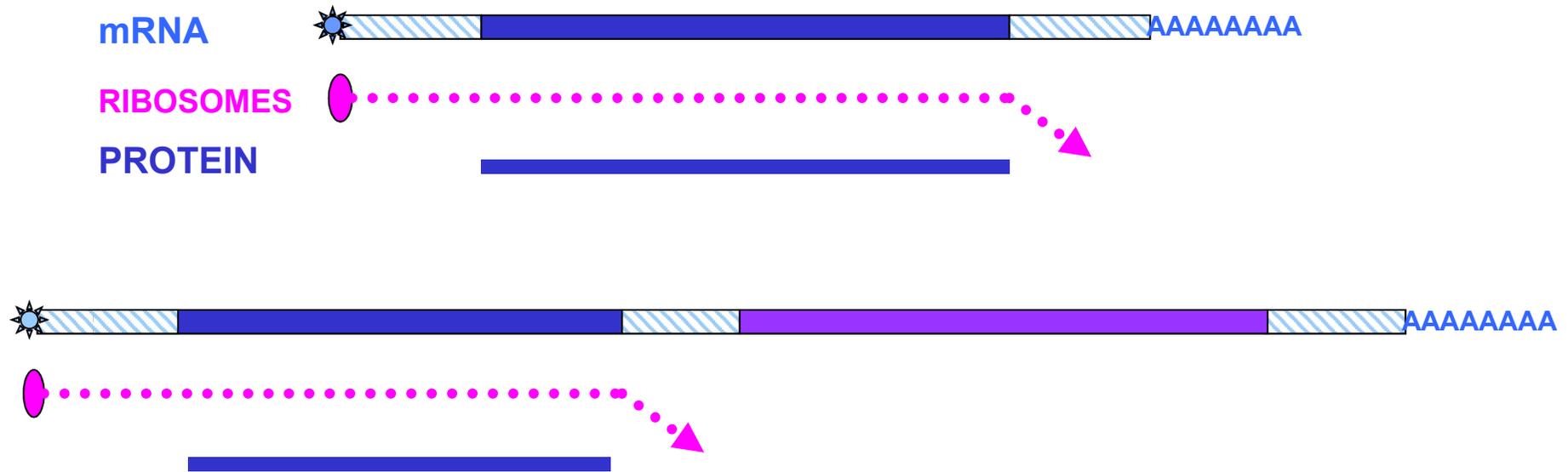
# RNA viruses that do NOT have a DNA phase

Genome	RNA-dependent RNA polymerase (=transcriptase) IN VIRION	Infectivity of RNA	Initial event in cell
Plus-stranded RNA	No	Infectious	Translation
Negative-stranded RNA	Yes	Non-infectious	Transcription
Double-stranded RNA	Yes	Non-infectious	Transcription

# RETROVIRUSES

<b>Genome</b>	<b>RNA-dependent DNA polymerase (=reverse transcriptase) in virion</b>	<b>Infectivity of RNA</b>	<b>Initial event in cell</b>
Plus-stranded RNA	Yes	Non-infectious	Reverse transcription

# The monocistronic mRNA problem



- Make one monocistronic mRNA per protein
- Make a primary transcript and use alternative splicing
- Make a large protein and then cut it into smaller proteins
- Include special features in the mRNA which enable ribosomes to bind internally

# GENOME SIZE

- TAUTOMERIZATION
- PROOF READING
  
- RNA VIRUSES HAVE SMALL GENOMES
  - ~10,000 NUCLEOTIDES
  - (herpesvirus DNA ~200,000)

# GENOME SIZE

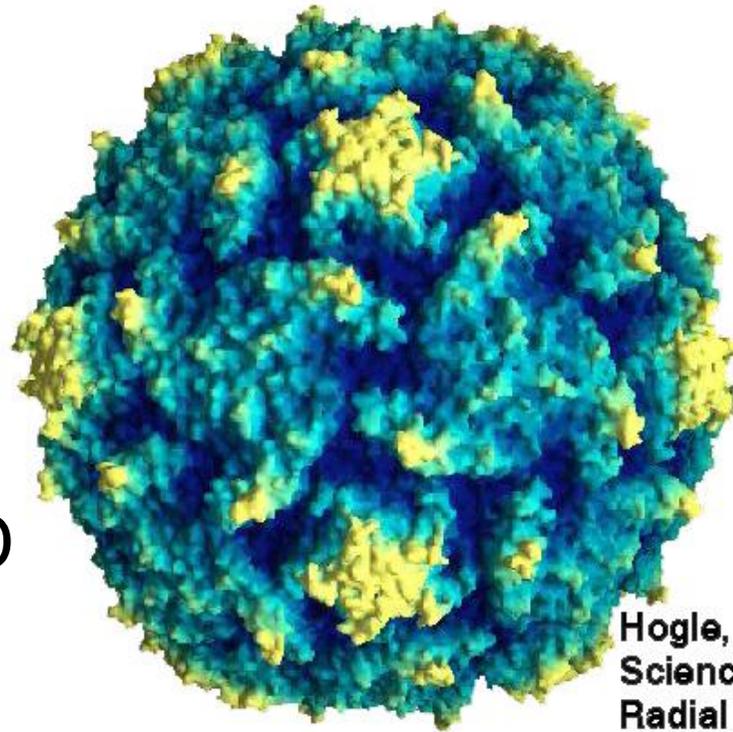
- POLYMERASE
- ATTACHMENT PROTEIN
- CAPSID PROTEIN
- RELATIVELY FEW OTHER PROTEINS
- VIRION SIZE MAY BE QUITE LARGE  
BUT LIMITED NUMBER OF  
PROTEINS

# POSITIVE-STRANDED RNA VIRUSES

- **EXAMPLES**
  - **PICORNAVIRUSES**
  - **TOGAVIRUSES**
  - **FLAVIVIRUSES**

# PICORNAVIRUS FAMILY (PICORNAVIRIDAE)

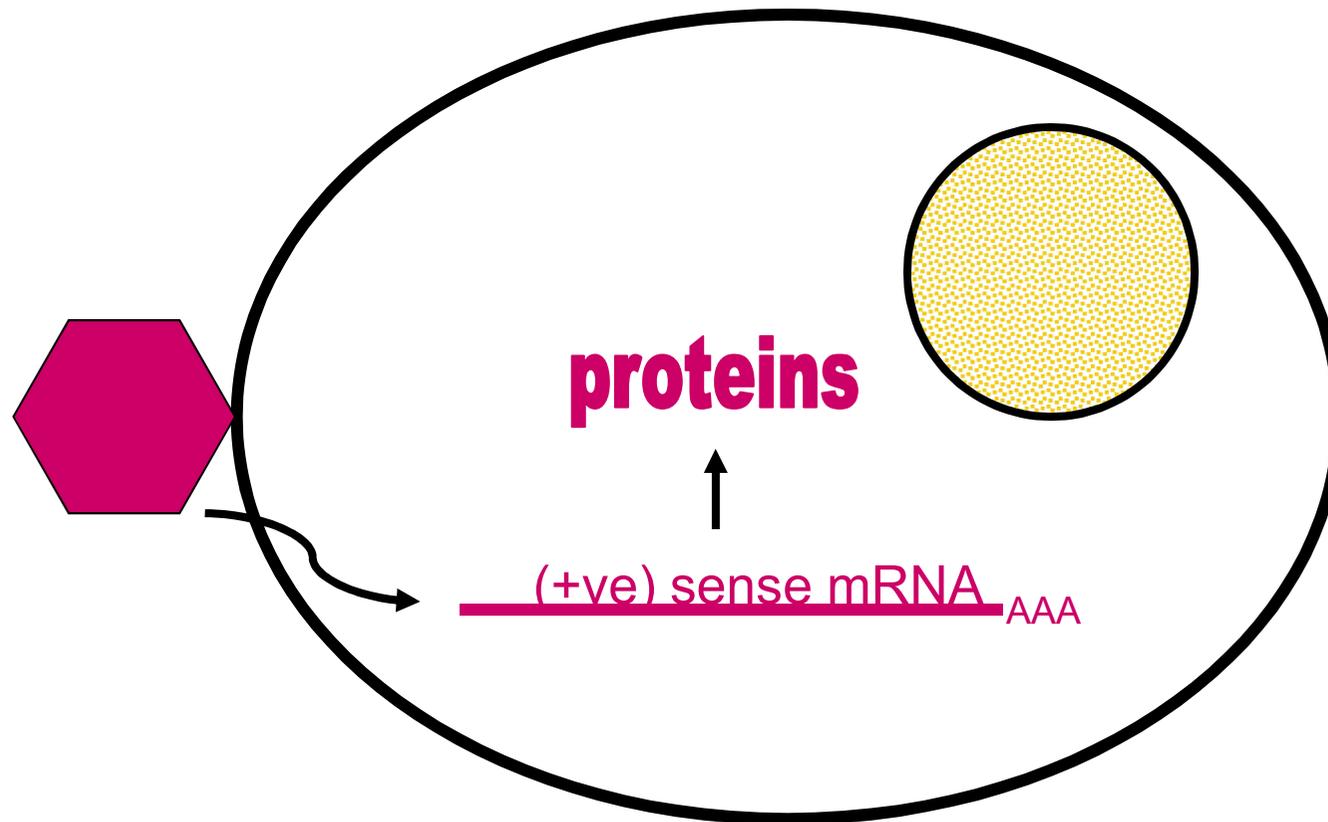
- SMALL
- ICOSAHEDRAL
- POSITIVE SENSE  
RNA
- NON-ENVELOPED



Poliovirus type 1

Hogle, Chow and Filman  
Science 229:1358  
Radial depth cue rendering  
J.Y.Sgro

# ADSORPTION AND PENETRATION



# INTERNAL RIBOSOME ENTRY SITE (IRES)



# Inhibition of host cell translation

Uninfected cell



Infected cell



# Viral translation

Uninfected cell



Infected cell



**GENOMIC (+ SENSE) RNA**

**AAAAA**

↓ translation

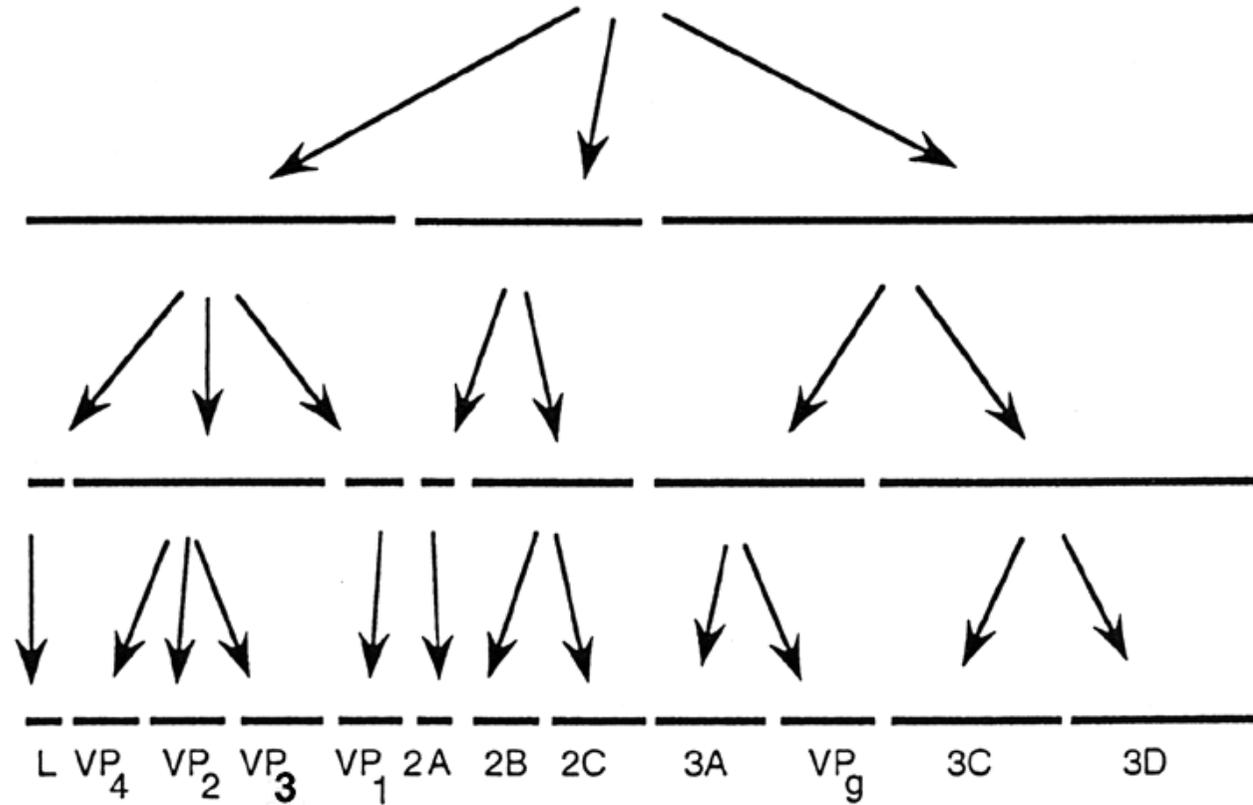
**POLYPROTEIN**

N END ————— C END

STEP 1

STEP 2

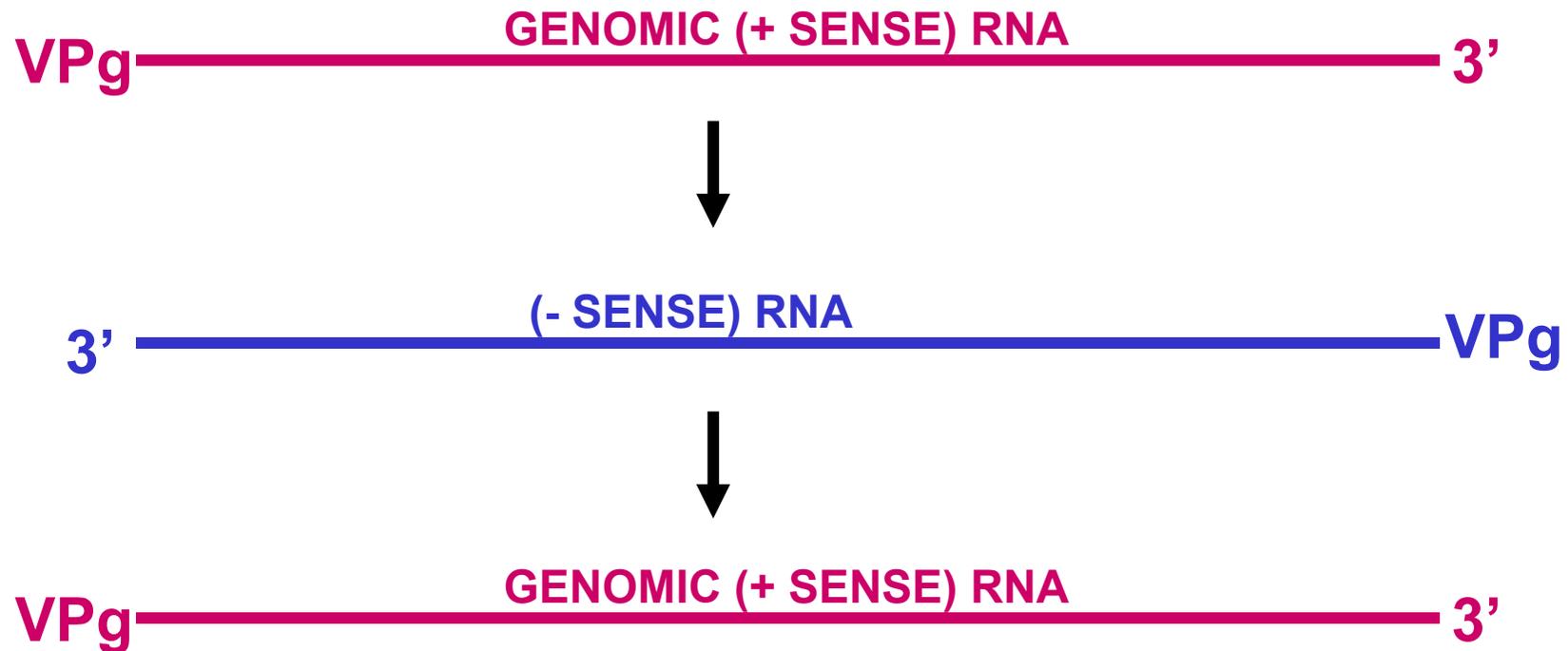
STEP 3



**CAPSID  
PROTEINS**

**PROTEASES,  
RNA SYNTHESIS**

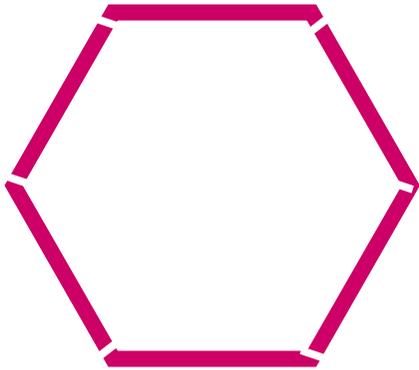
# RNA REPLICATION



# RNA REPLICATION

- viral RNA polymerase (replicase)
- host factors also involved as accessory proteins
- new plus strands
  - packaged
  - templates for more replication
  - templates for more translation

# ASSEMBLY



**VP0, VP1, VP3**



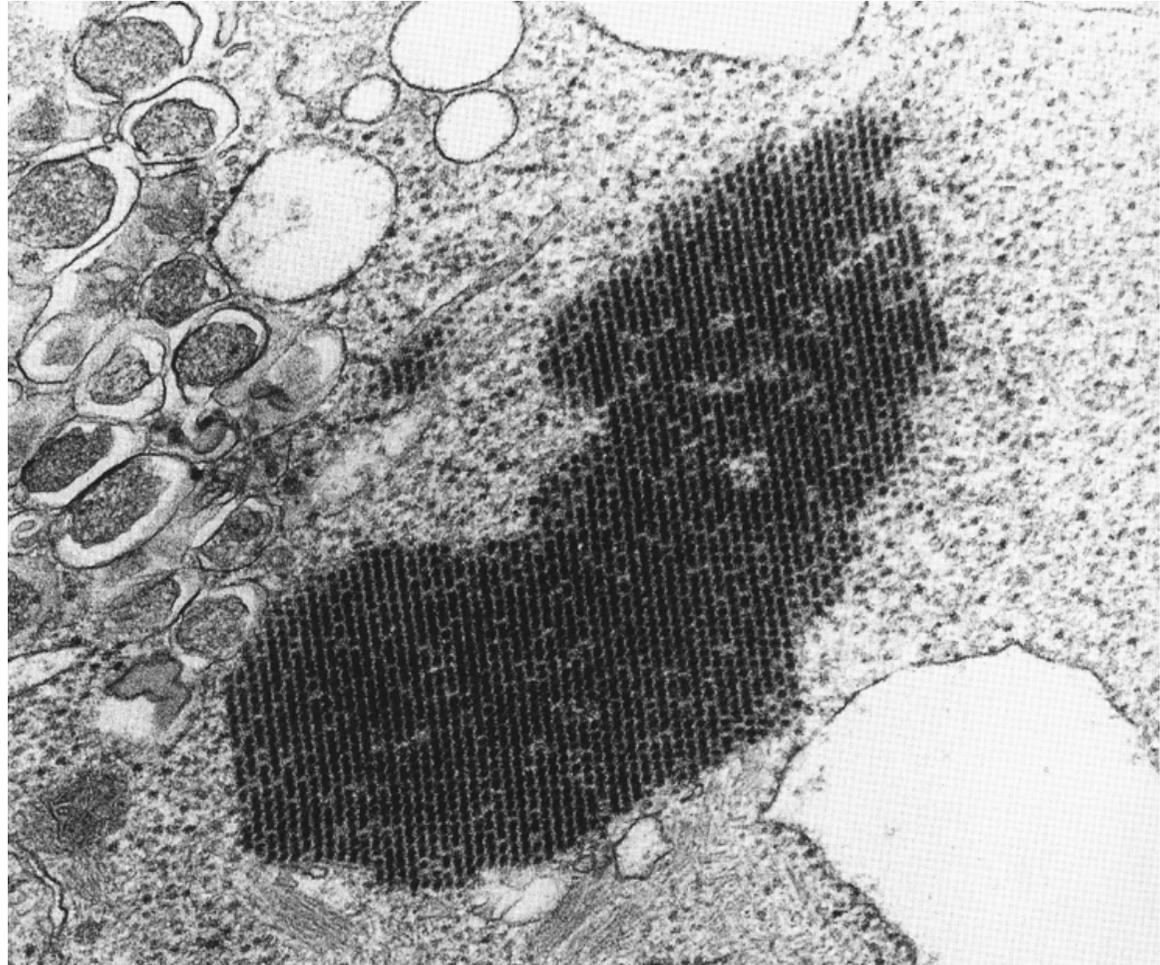
**VPg-RNA enters, VP0 is cleaved**



**VP2, VP4, VP1, VP3**

# RELEASE

- LYSIS



# NON-SEGMENTED NEGATIVE STRAND VIRUSES

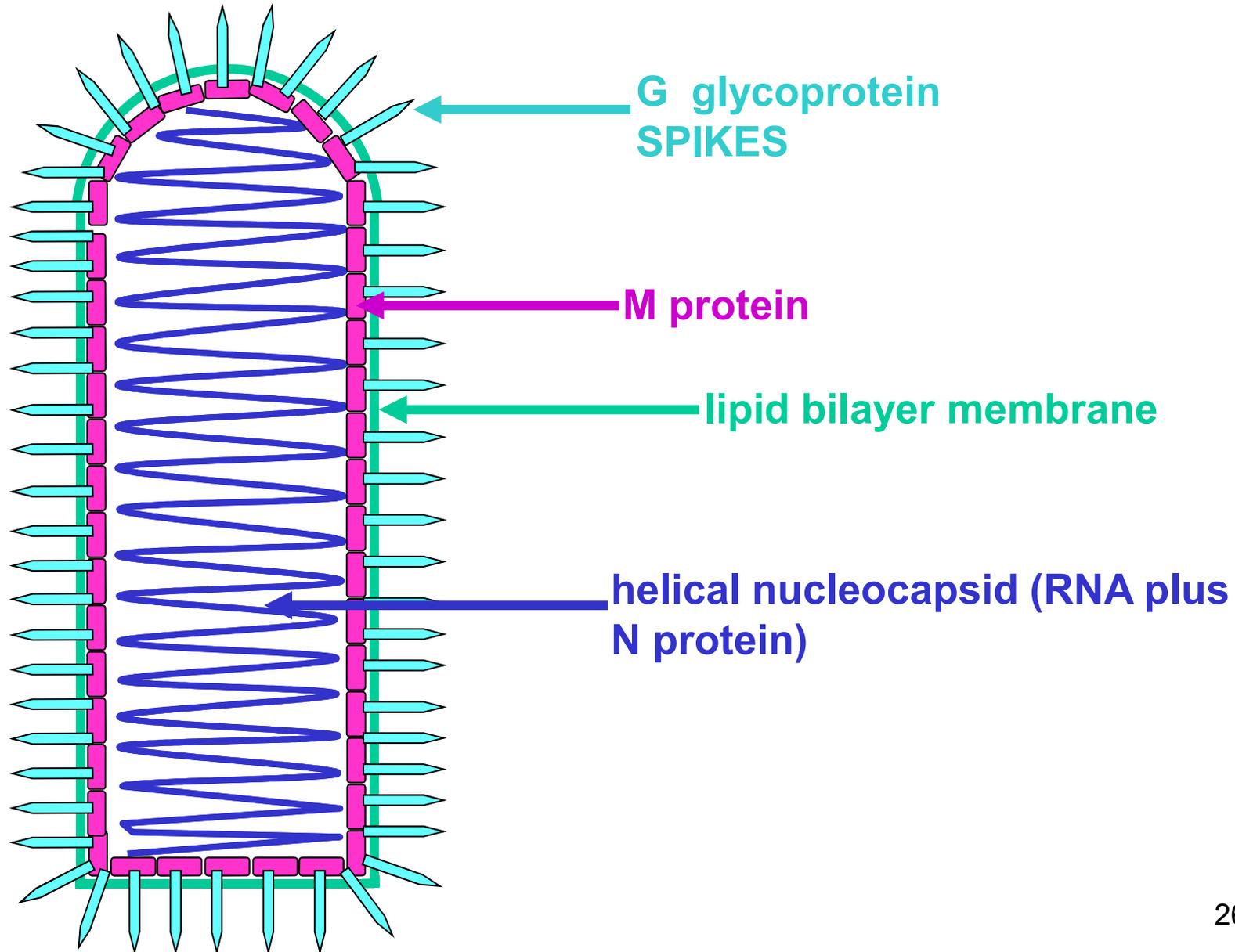
**Examples:**

**Rhabdovirus family (Rhabdoviridae)**

**Paramyxovirus family (Paramyxoviridae)**

**Filovirus family (Filoviridae)**

# RHABDOVIRUSES

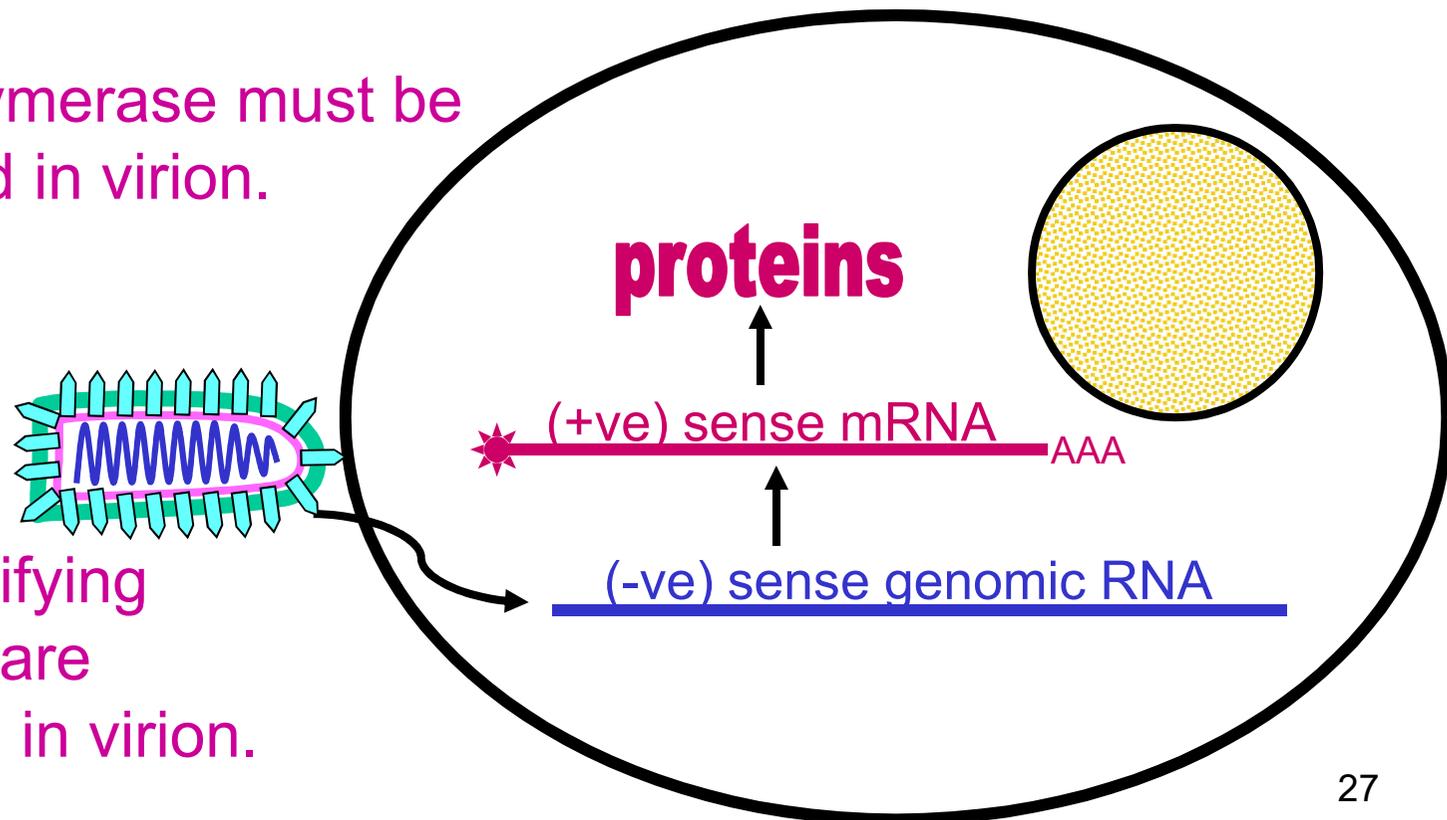


# Need to make mRNA

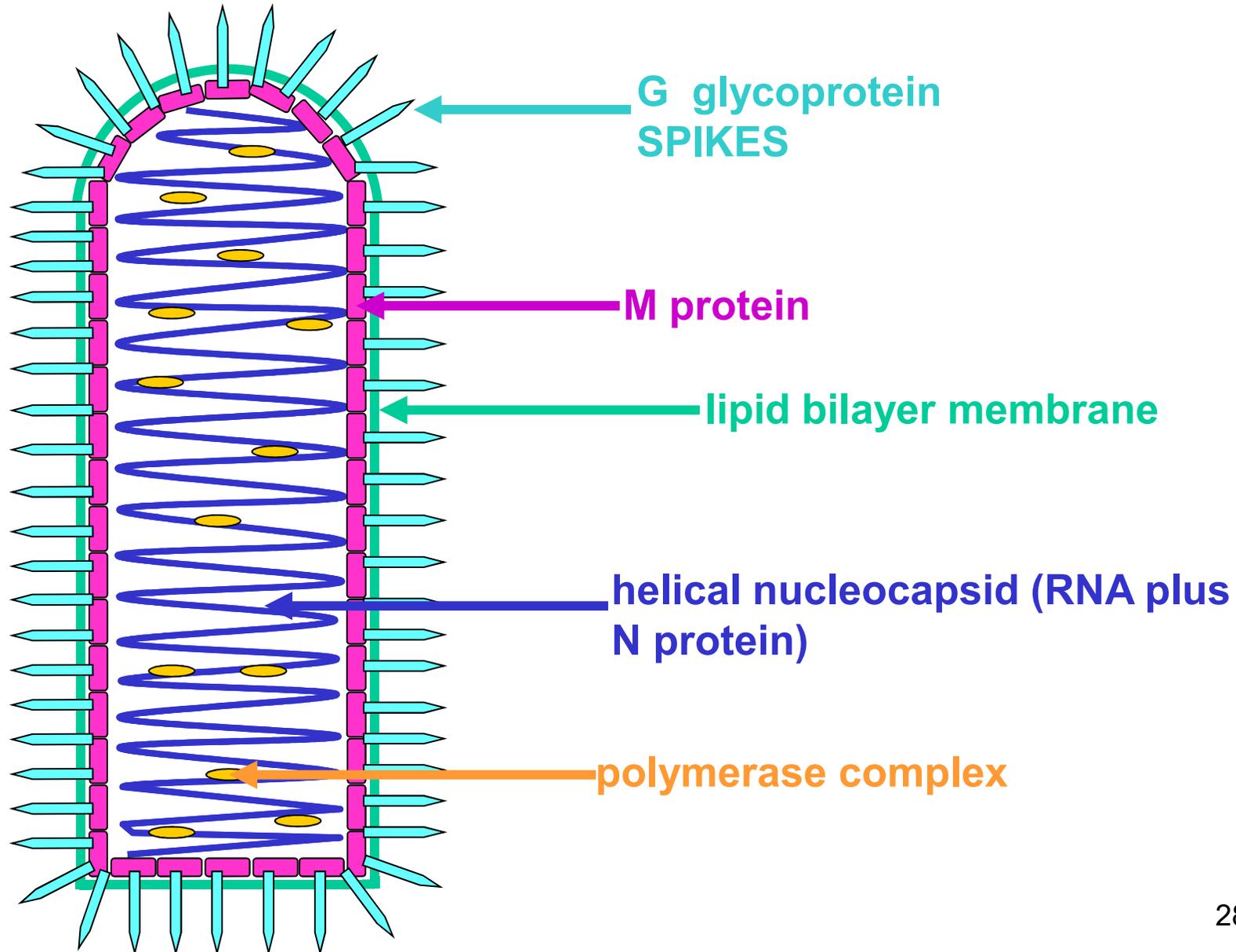
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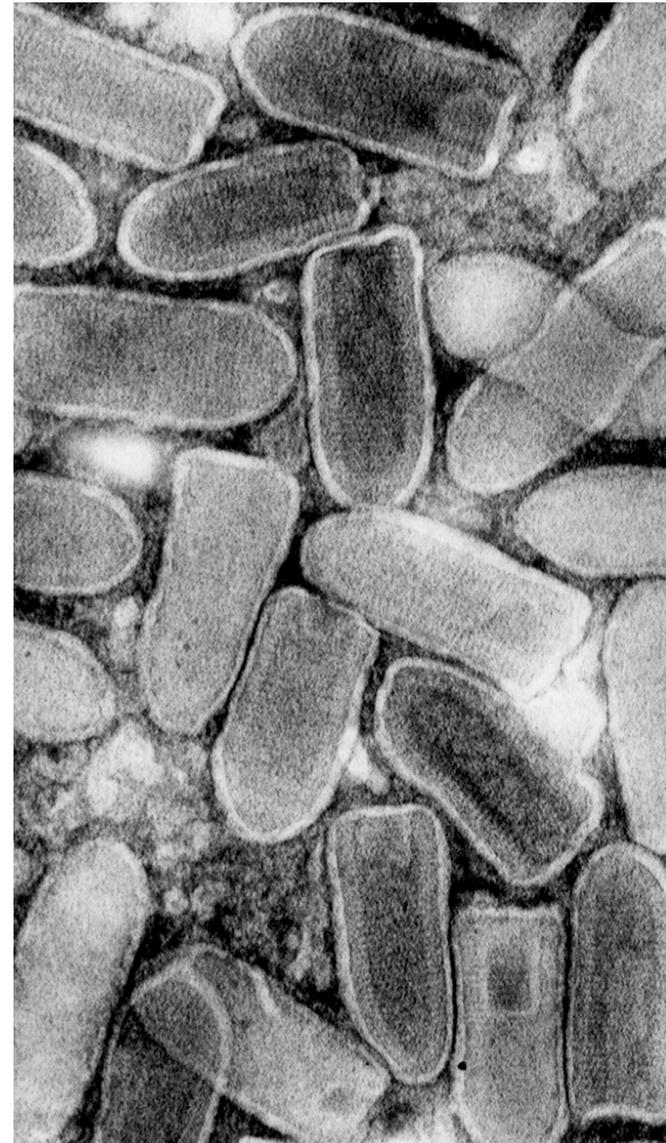


# RHABDOVIRUSES

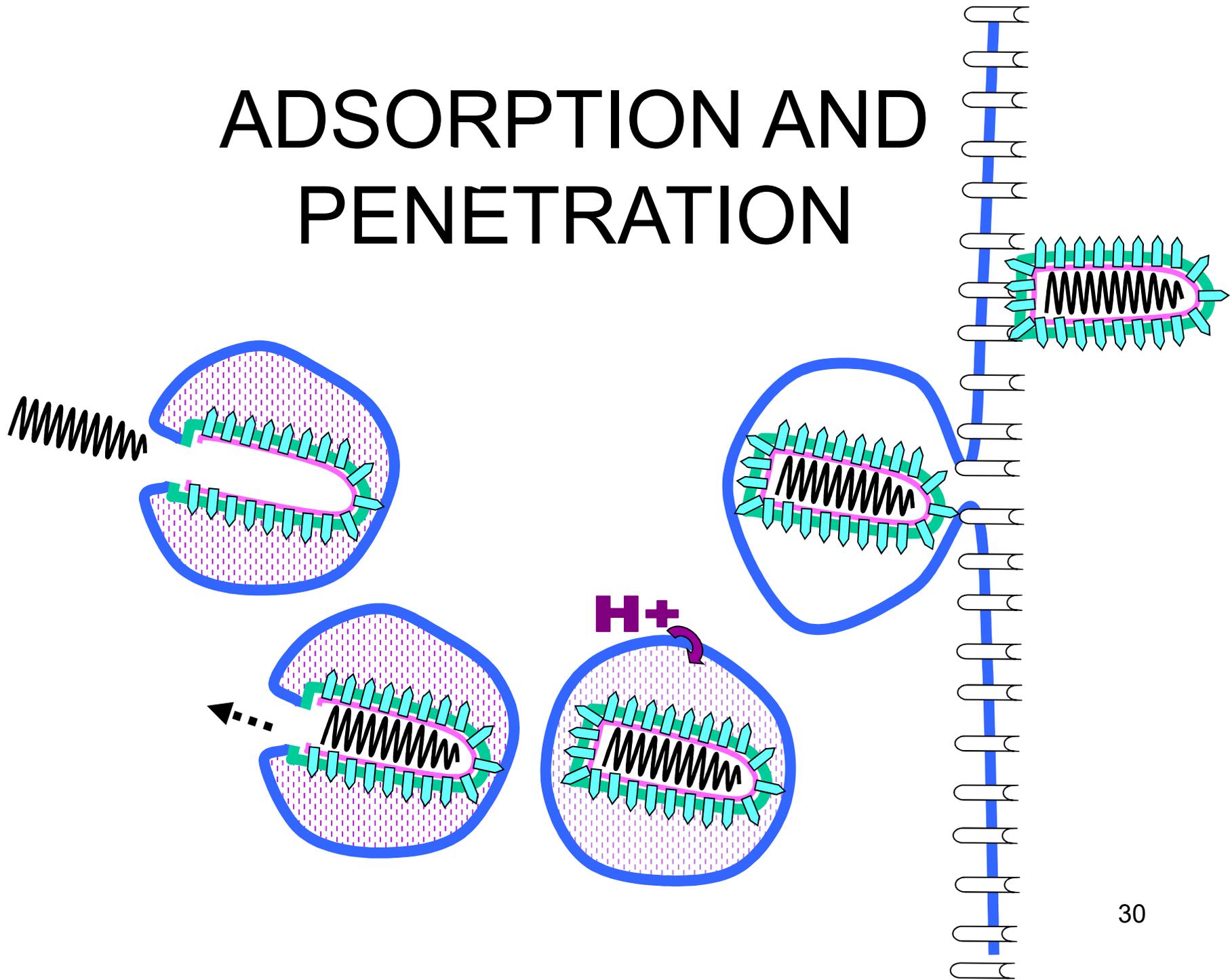


# RHABDOVIRUSES

- EXAMPLES
  - vesicular stomatitis virus (VSV)
  - rabies virus



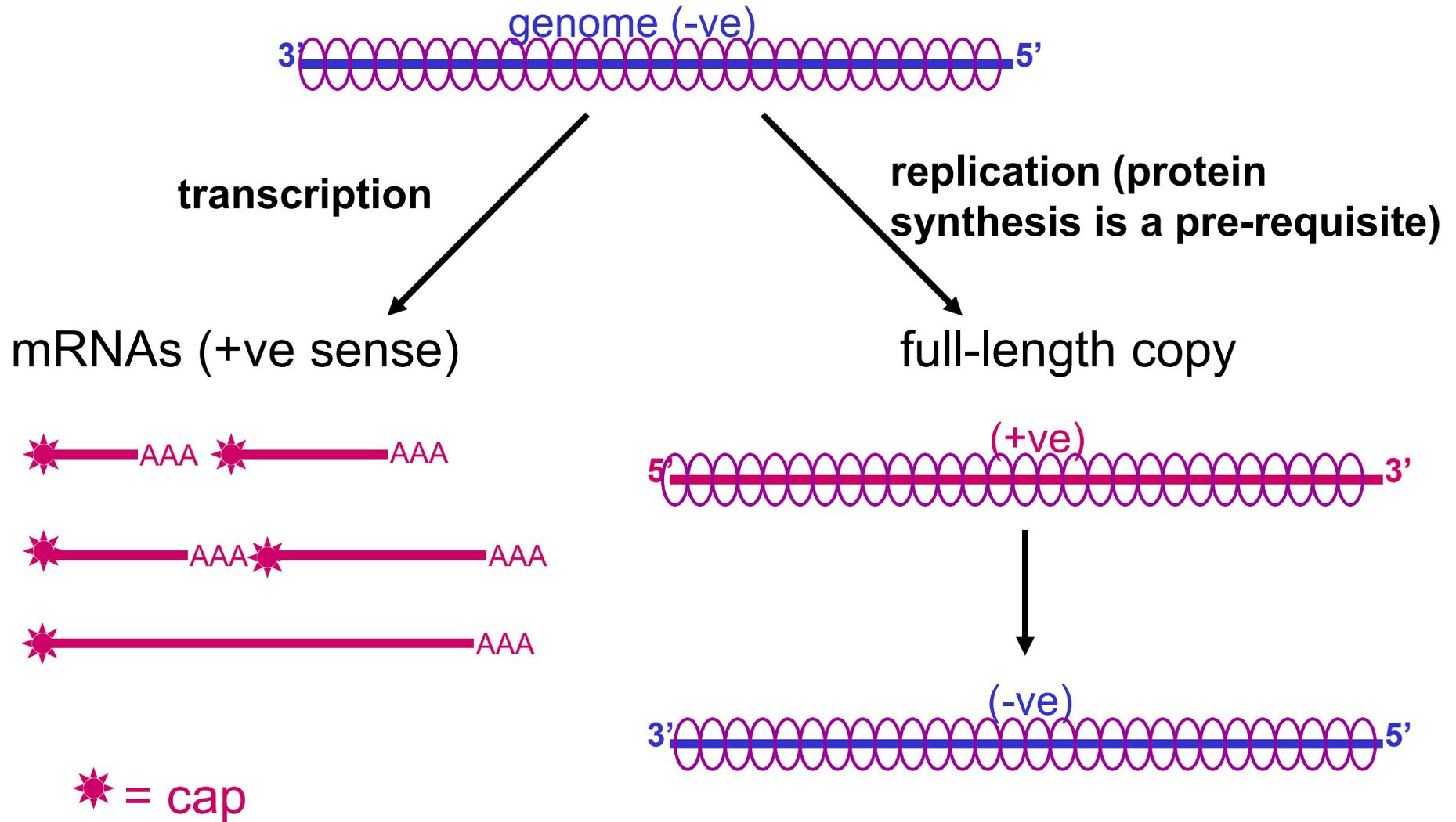
# ADSORPTION AND PENETRATION



# ADSORPTION AND PENETRATION

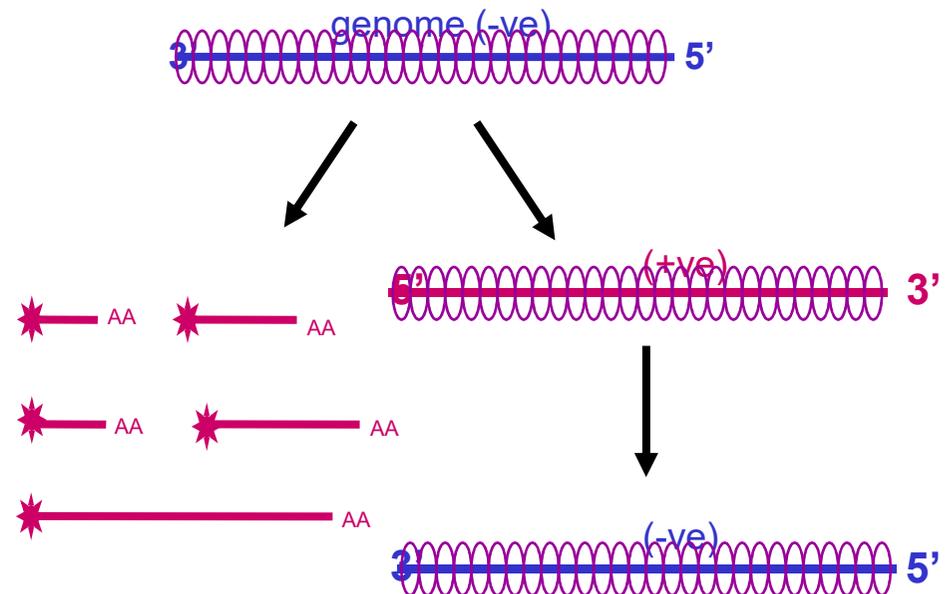
- CYTOPLASMIC REPLICATION
- GENOMIC RNA REMAINS IN  
NUCLEOCAPSID FORM

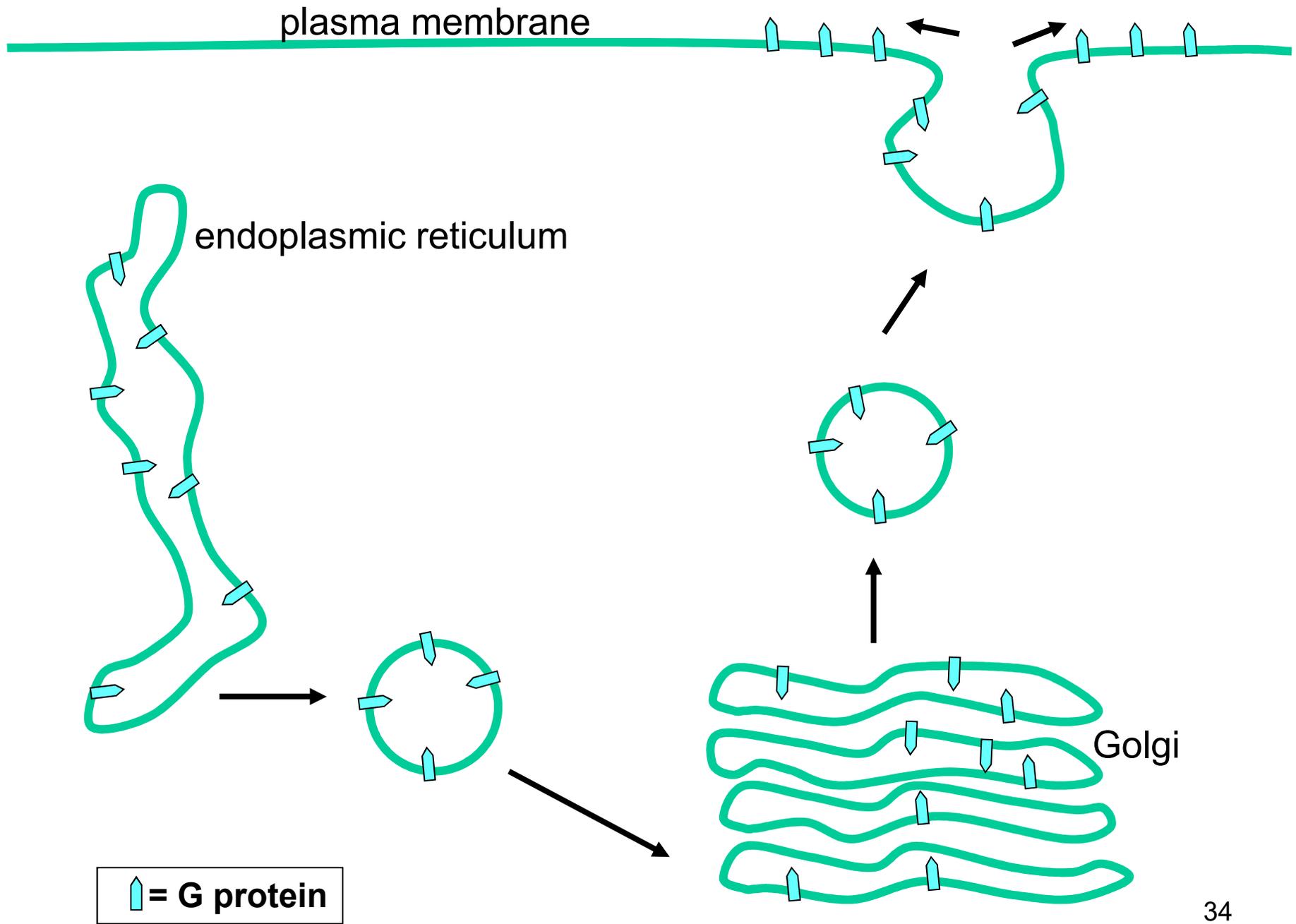
# RNA SYNTHESIS



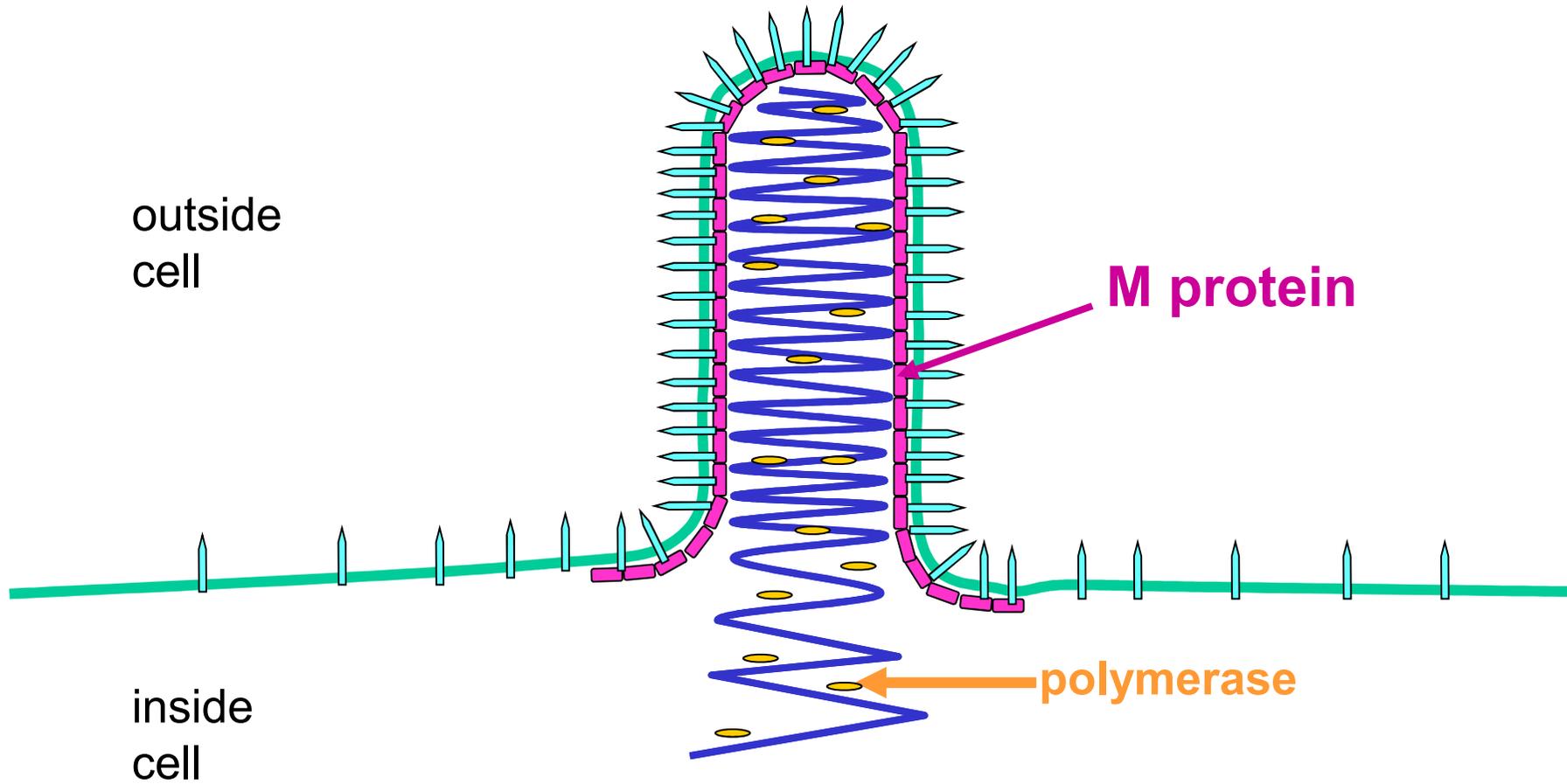
# NEW MINUS STRAND

- PACKAGED
- TEMPLATE FOR MORE REPLICATION
- TEMPLATE FOR MORE TRANSCRIPTION





# ASSEMBLY



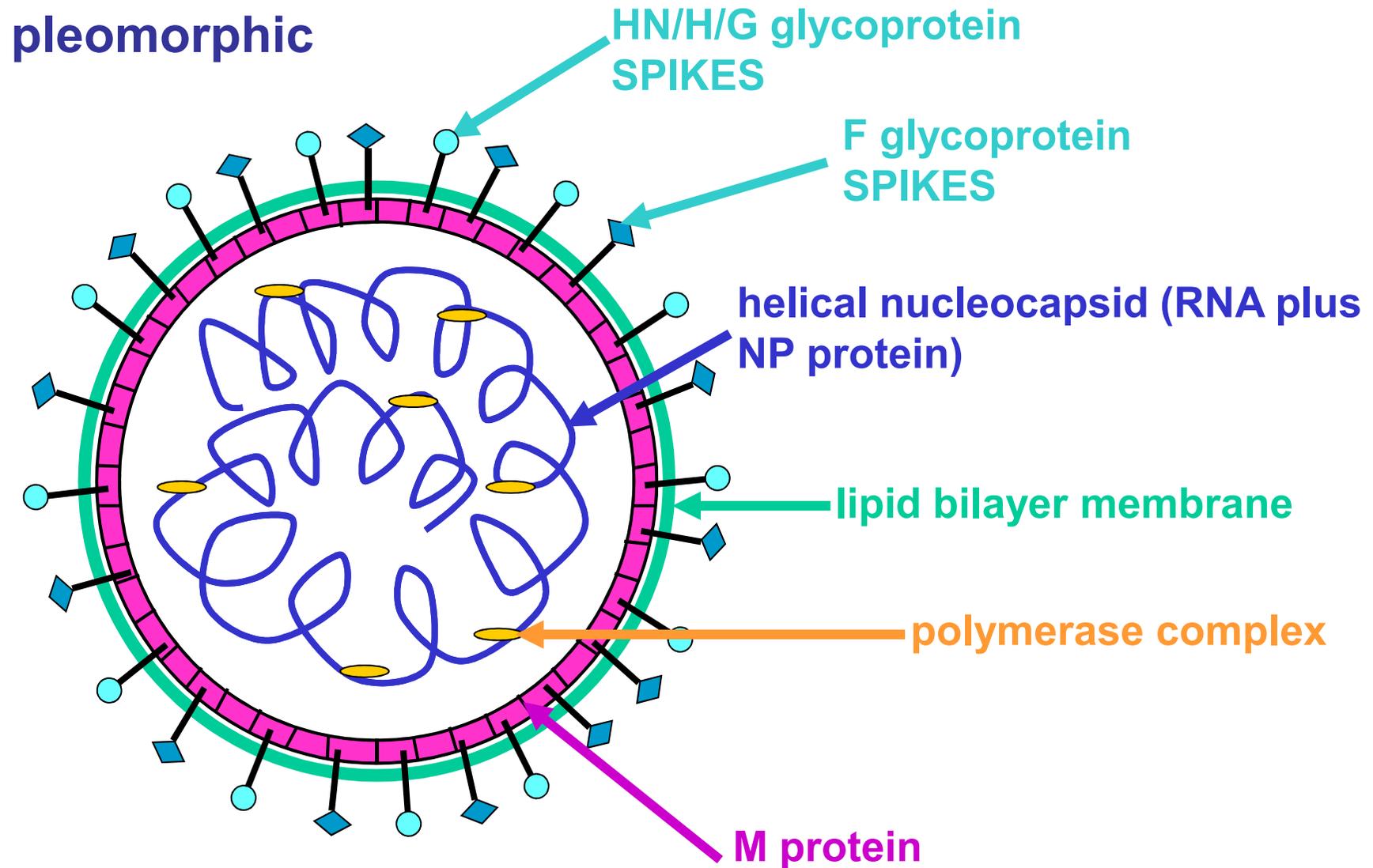
# SOME POINTS TO NOTE ABOUT RHABDOVIRUSES

- ENTIRE CYCLE OCCURS IN CYTOPLASM
- RNA POLYMERASE AND RNA MODIFICATION ENZYMES ARE:
  - VIRALLY CODED
  - PRESENT IN THE VIRION
- THERE IS NO EARLY/LATE DIVISION

# PARAMYXOVIRUSES

- MEMBERS INCLUDE
  - PARAINFLUENZA VIRUS
  - MUMPS VIRUS
  - MEASLES VIRUS
  - RESPIRATORY SYNCYTIAL VIRUS

# PARAMYXOVIRUSES



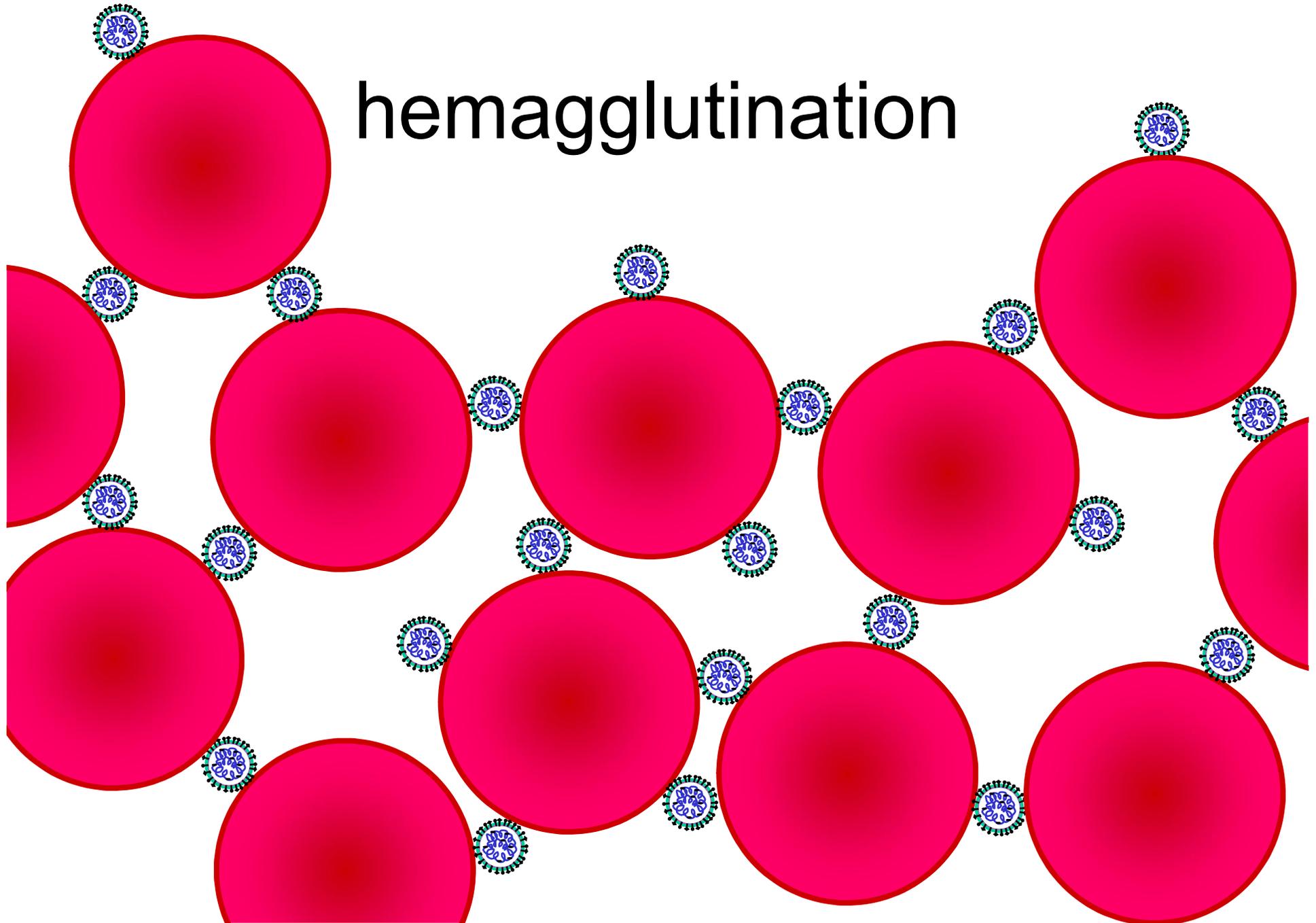
# PARAMYXOVIRIDAE

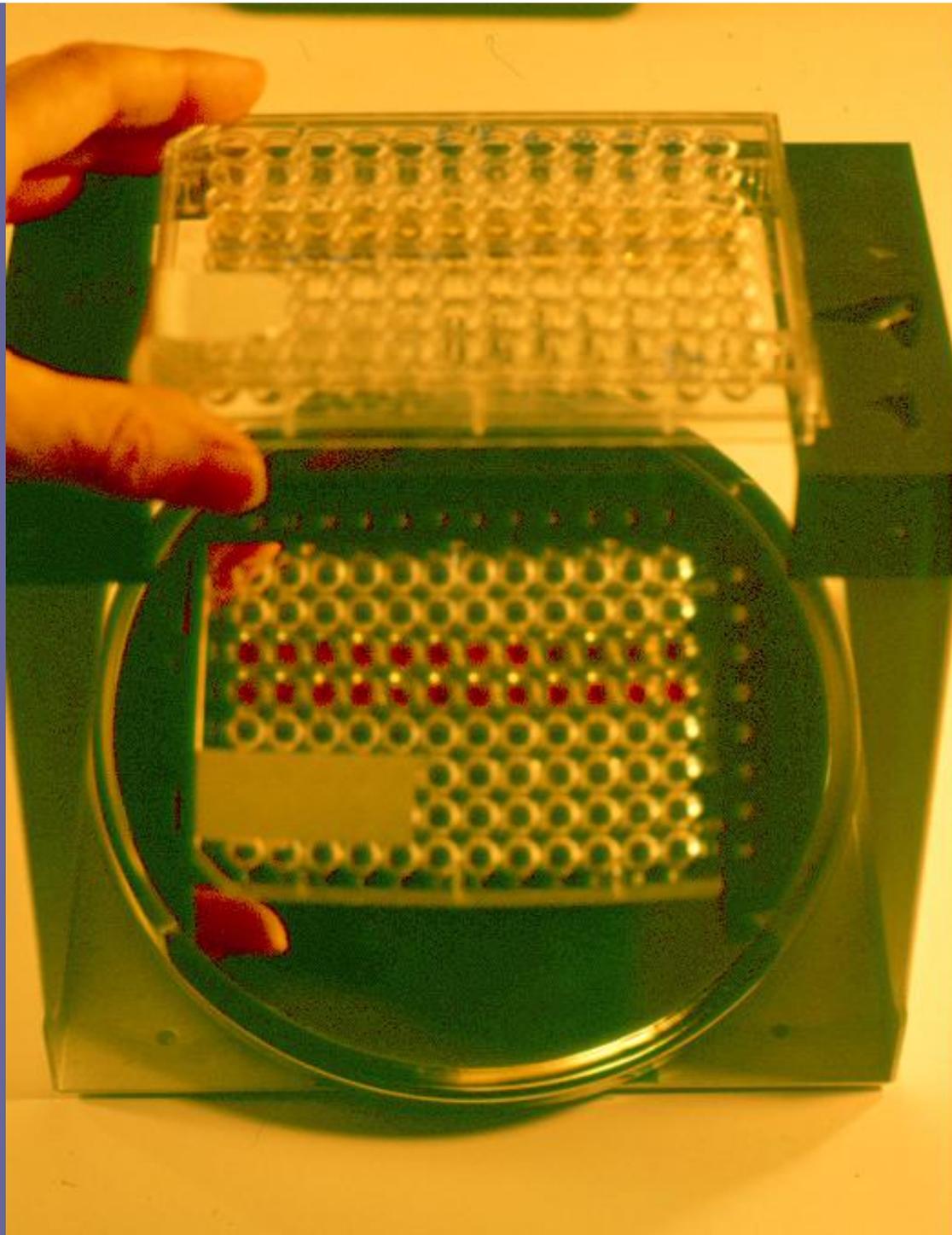
- PARAMYXOVIRUS SUBFAMILY
  - PARAMYXOVIRUS
  - RUBULAVIRUS
  - MORBILLIVIRUS
- PNEUMOVIRUS SUBFAMILY
  - PNEUMOVIRUS GENUS
  - METAPNEUMOVIRUS GENUS

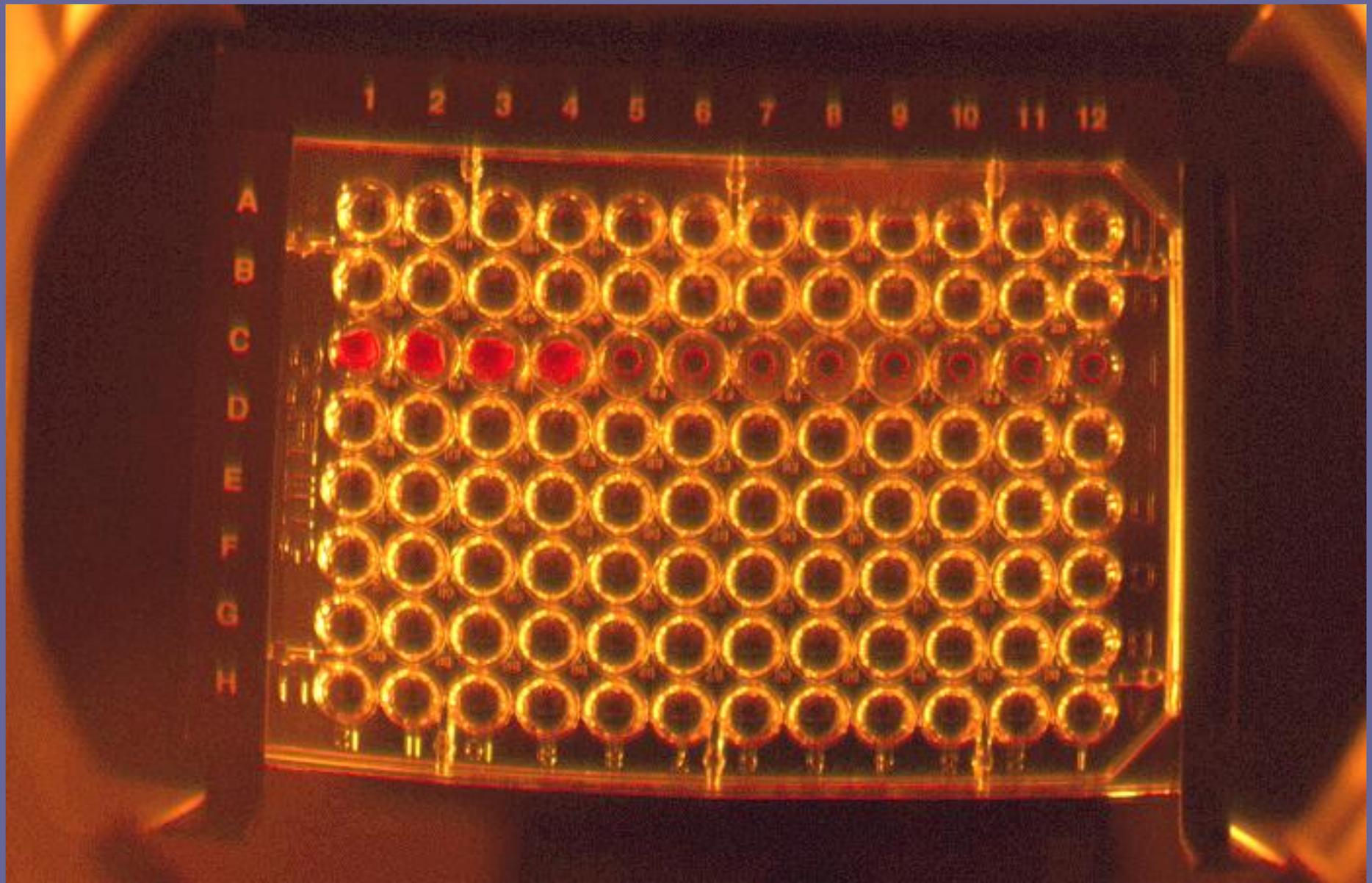
## PARAMYXOVIRUS FAMILY SURFACE GLYCOPROTEINS

GENUS	GLYCOPROTEINS	TYPICAL MEMBERS
<b>PARAMYXOVIRUS SUBFAMILY</b>		
Paramyxovirus	HN, F	HPIV1, HPIV3
Rubulavirus	HN, F	HPIV2, HPIV4, mumps virus
Morbillivirus	H, F	measles virus
<b>PNEUMOVIRUS SUBFAMILY</b>		
Pneumovirus	G, F	respiratory syncytial virus
Metapneumovirus	G, F	metapneumoviruses

# hemagglutination

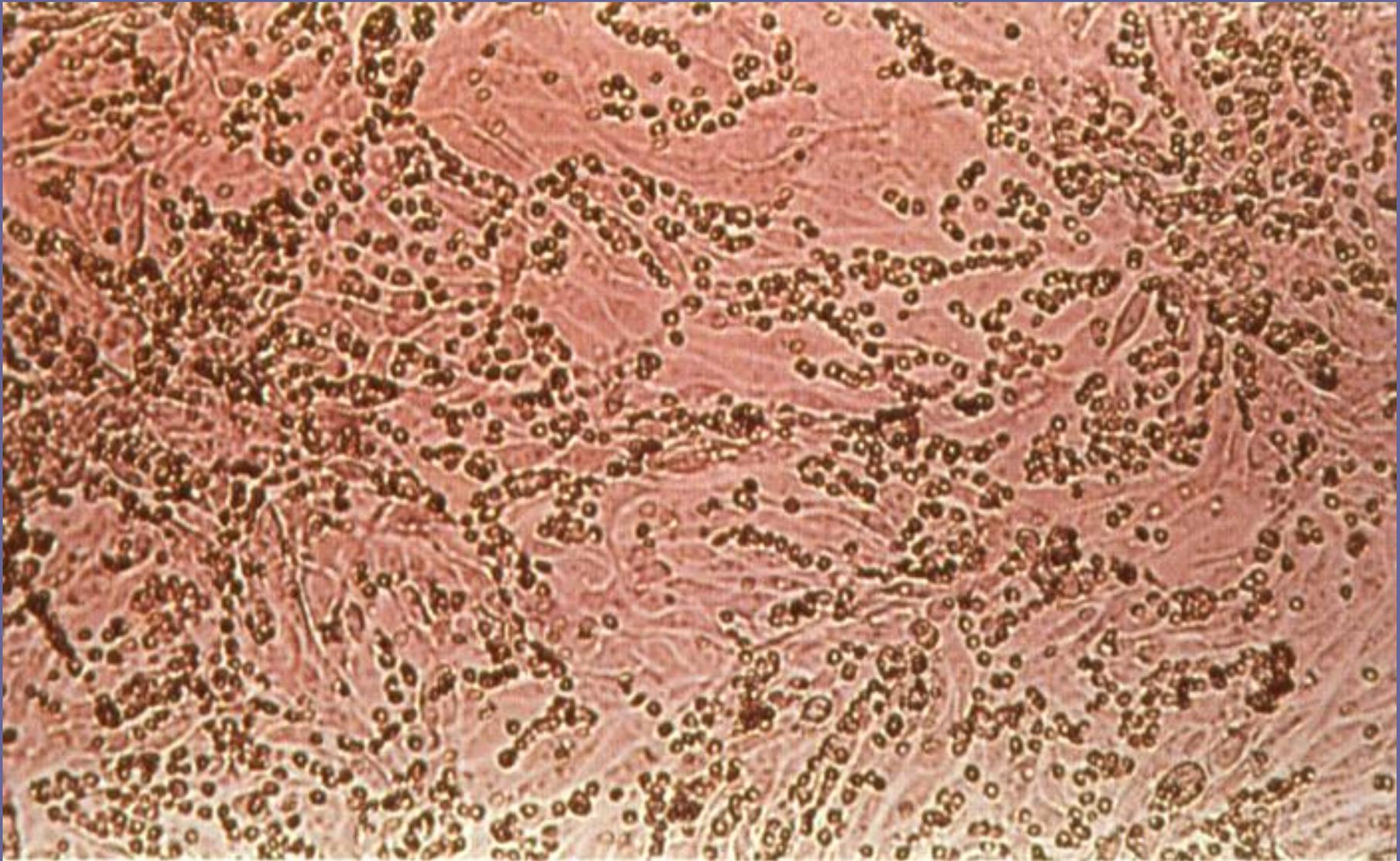






# HEMAGGLUTINATION

- Quick, easy, cheap
- Can use to detect virus or antibody to virus (hemagglutination inhibition)
- A measure of total virus particles present, not of infectious virus particles



Hemadsorption - influenza virus infected cells

# PARAMYXOVIRUS FAMILY

## PROPERTIES OF THE ATTACHMENT PROTEIN

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# Neuraminidase activity

- Destroys sialic acid/neuraminic acid

# PARAMYXOVIRUS FAMILY

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# PARAMYXOVIRUS FAMILY

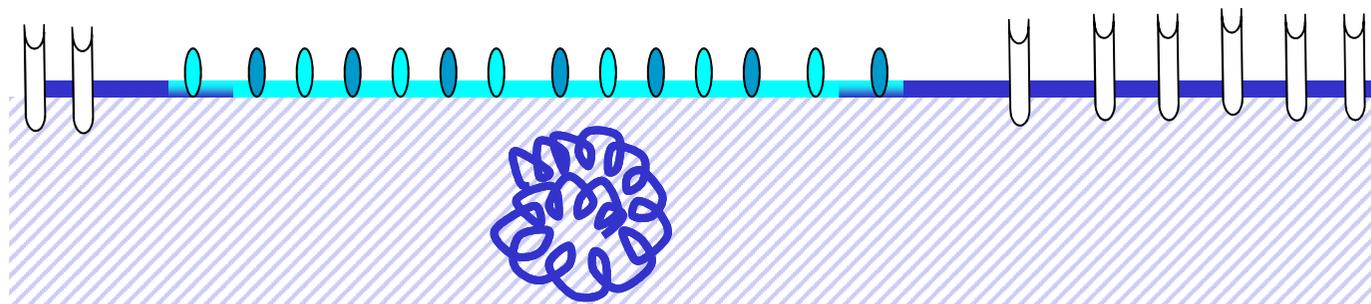
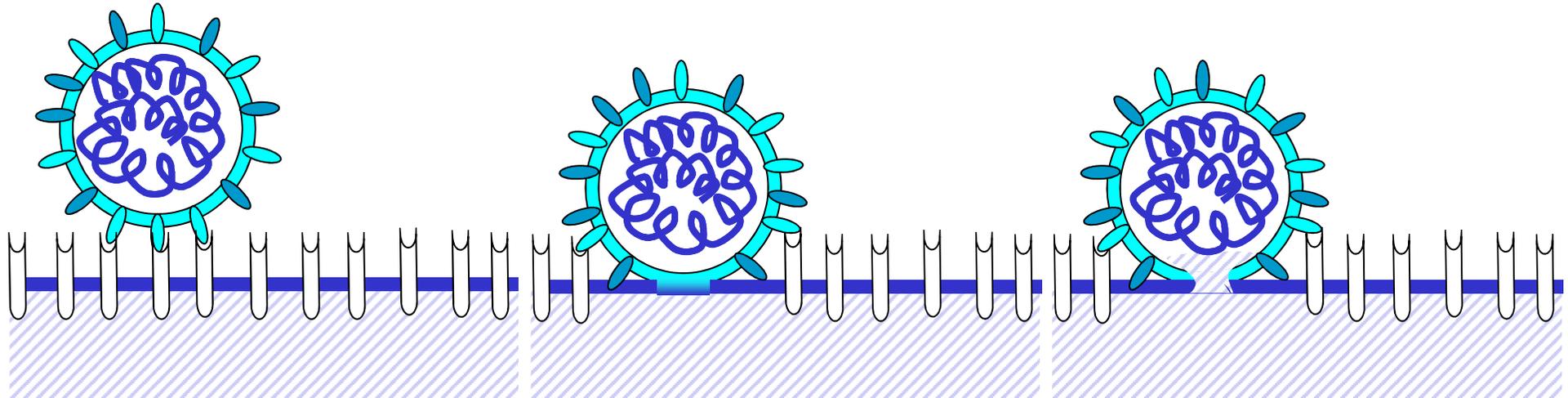
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# F PROTEIN

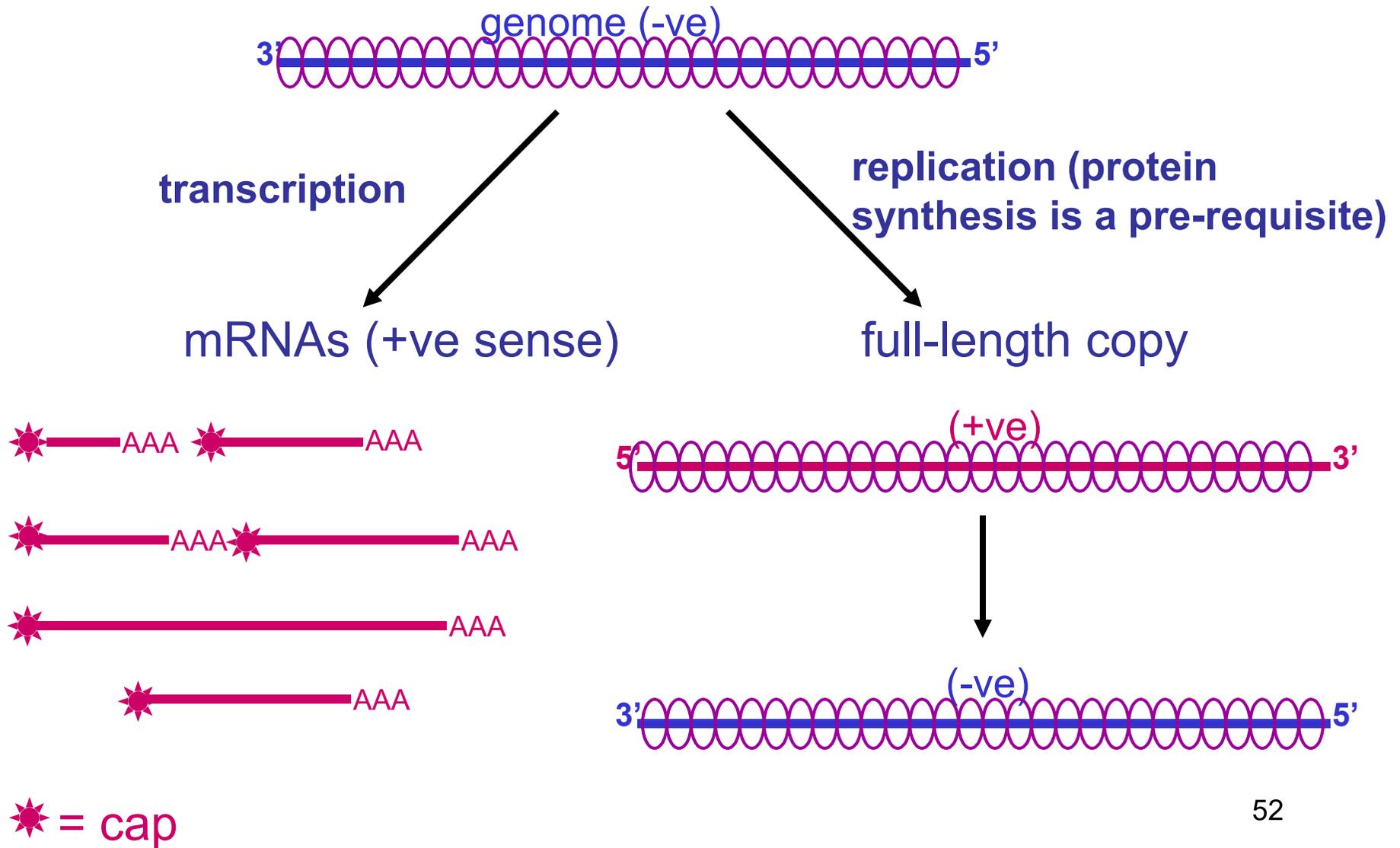
- Fusion
- Works at physiological pH

# PENETRATION - FUSION

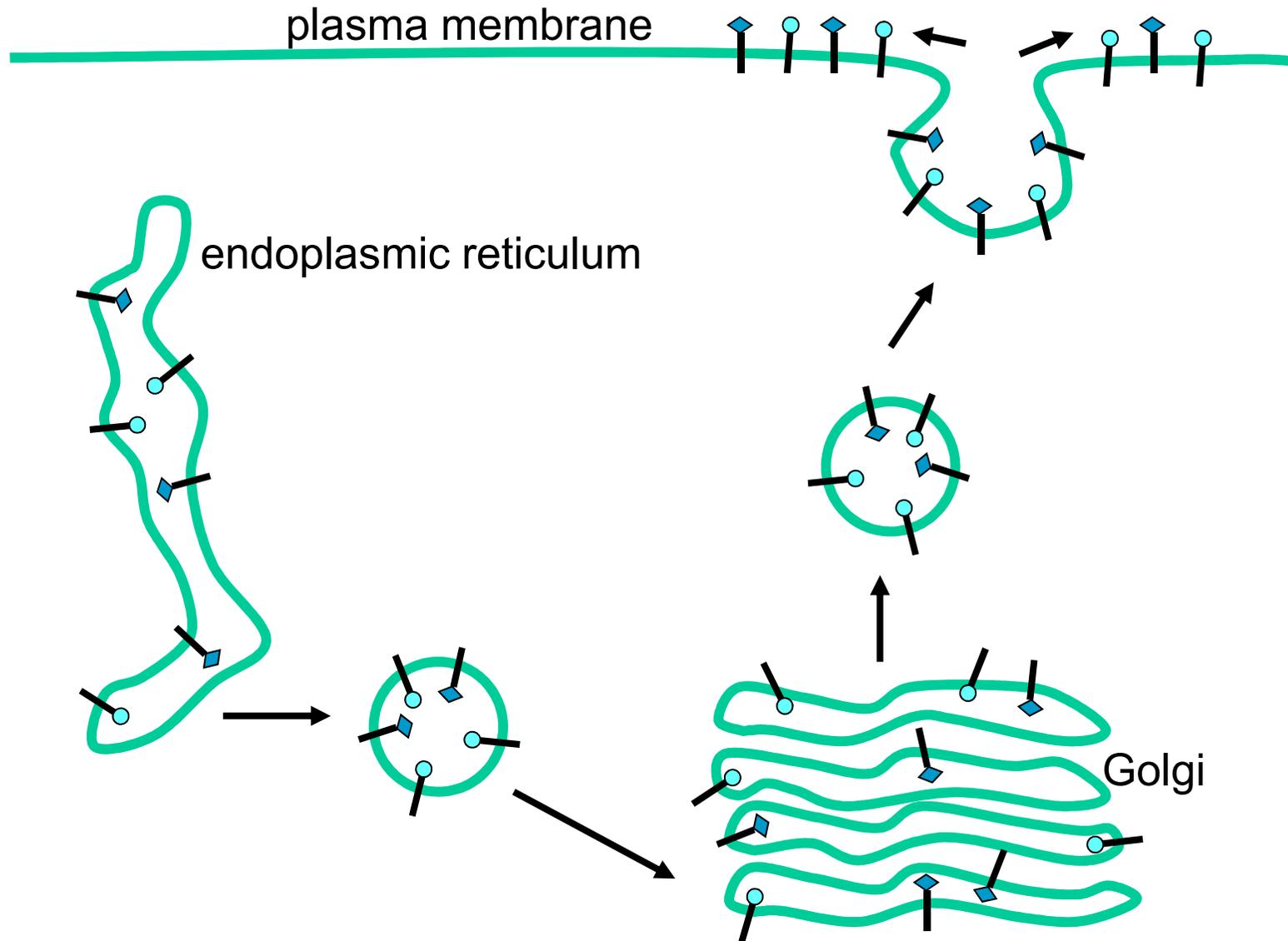


replicates in cytoplasm

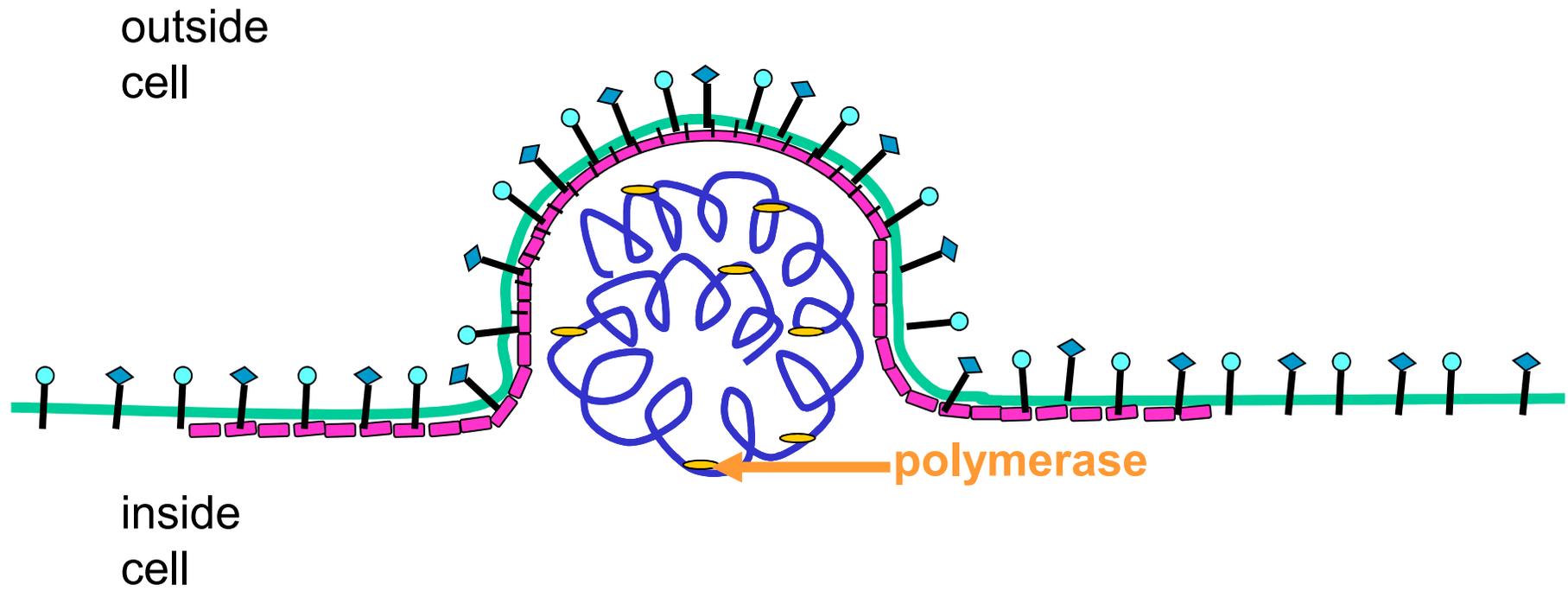
# TRANSCRIPTION, TRANSLATION, REPLICATION



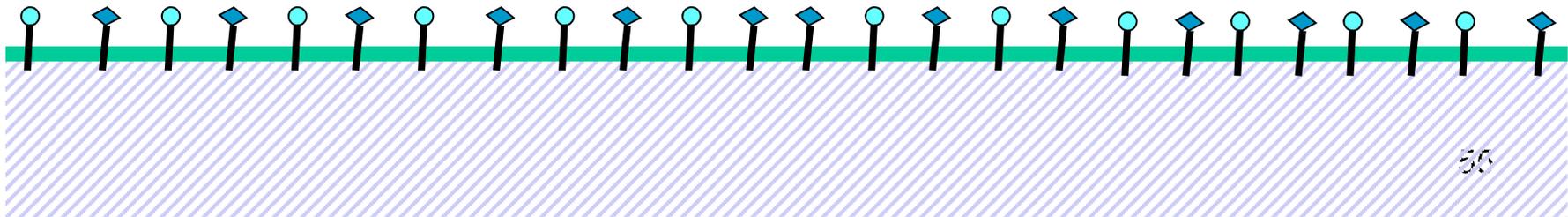
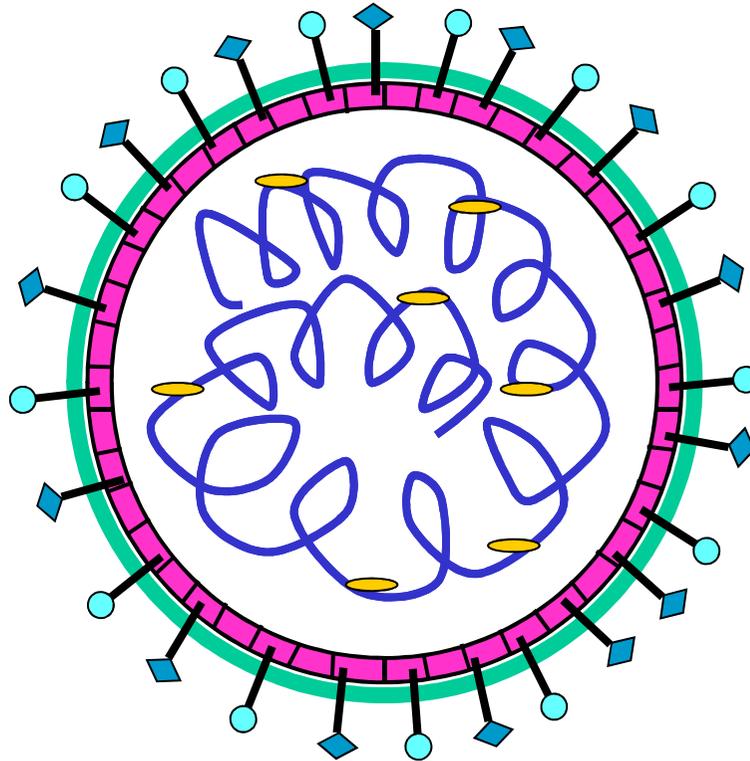
# ASSEMBLY



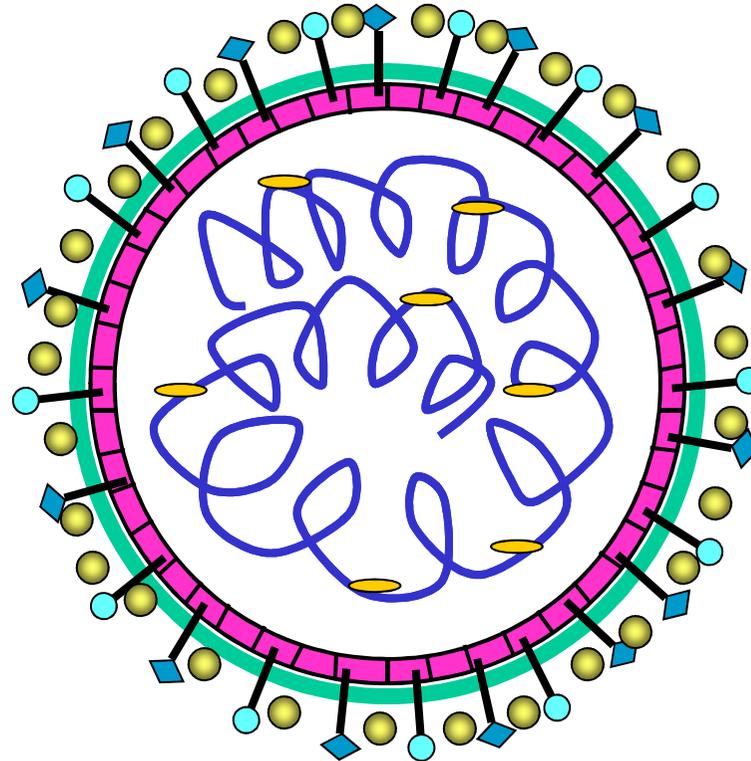
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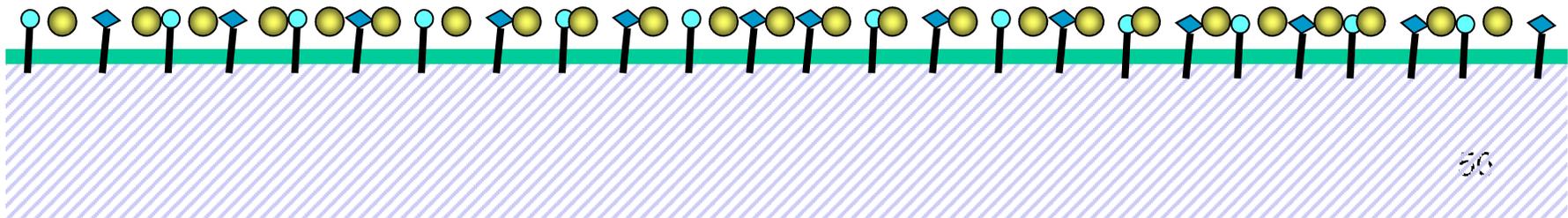
# ROLE OF NEURAMINIDASE



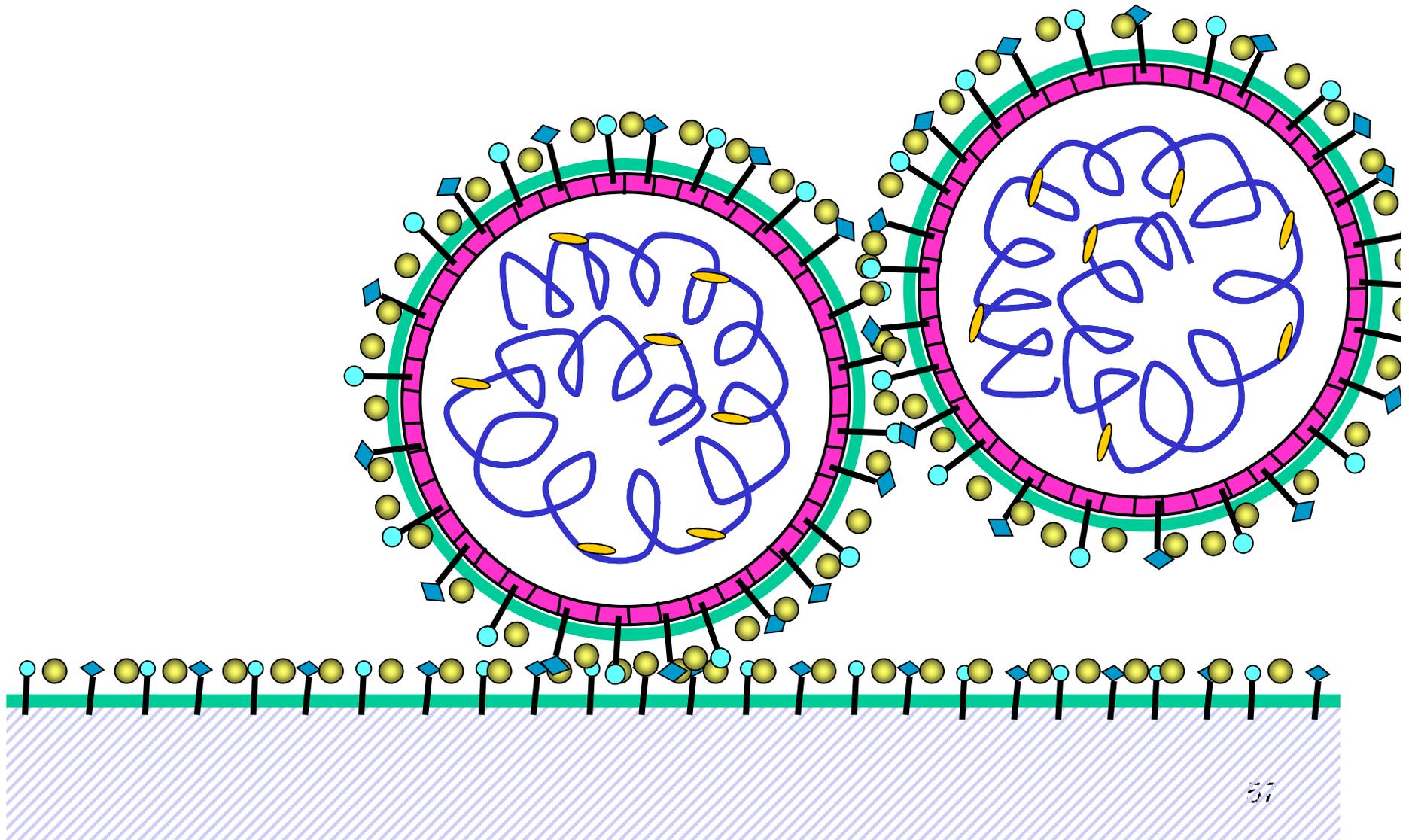
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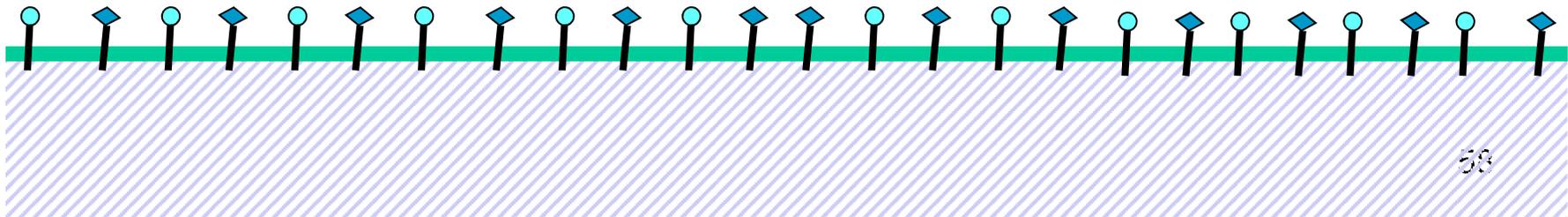
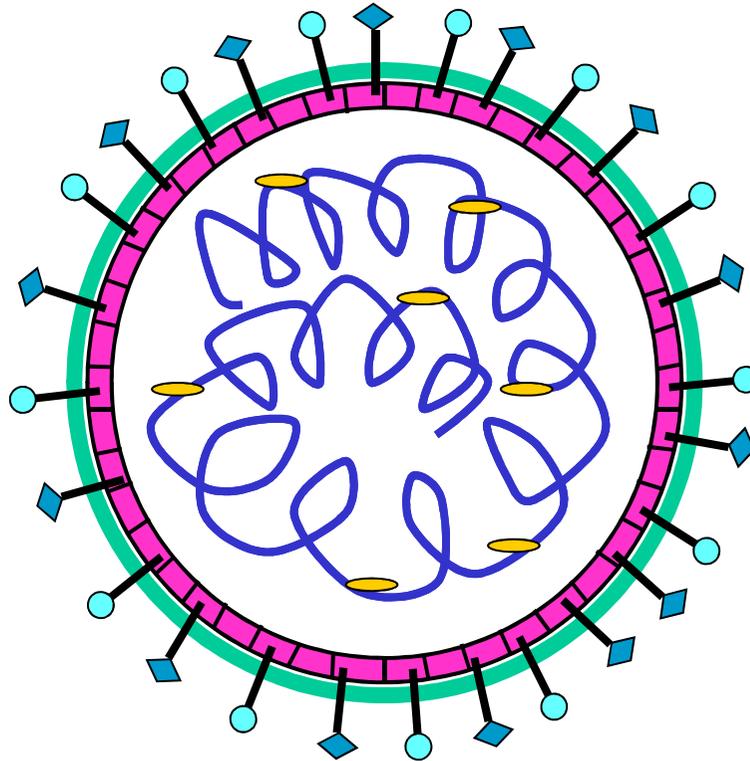
●=sialic acid



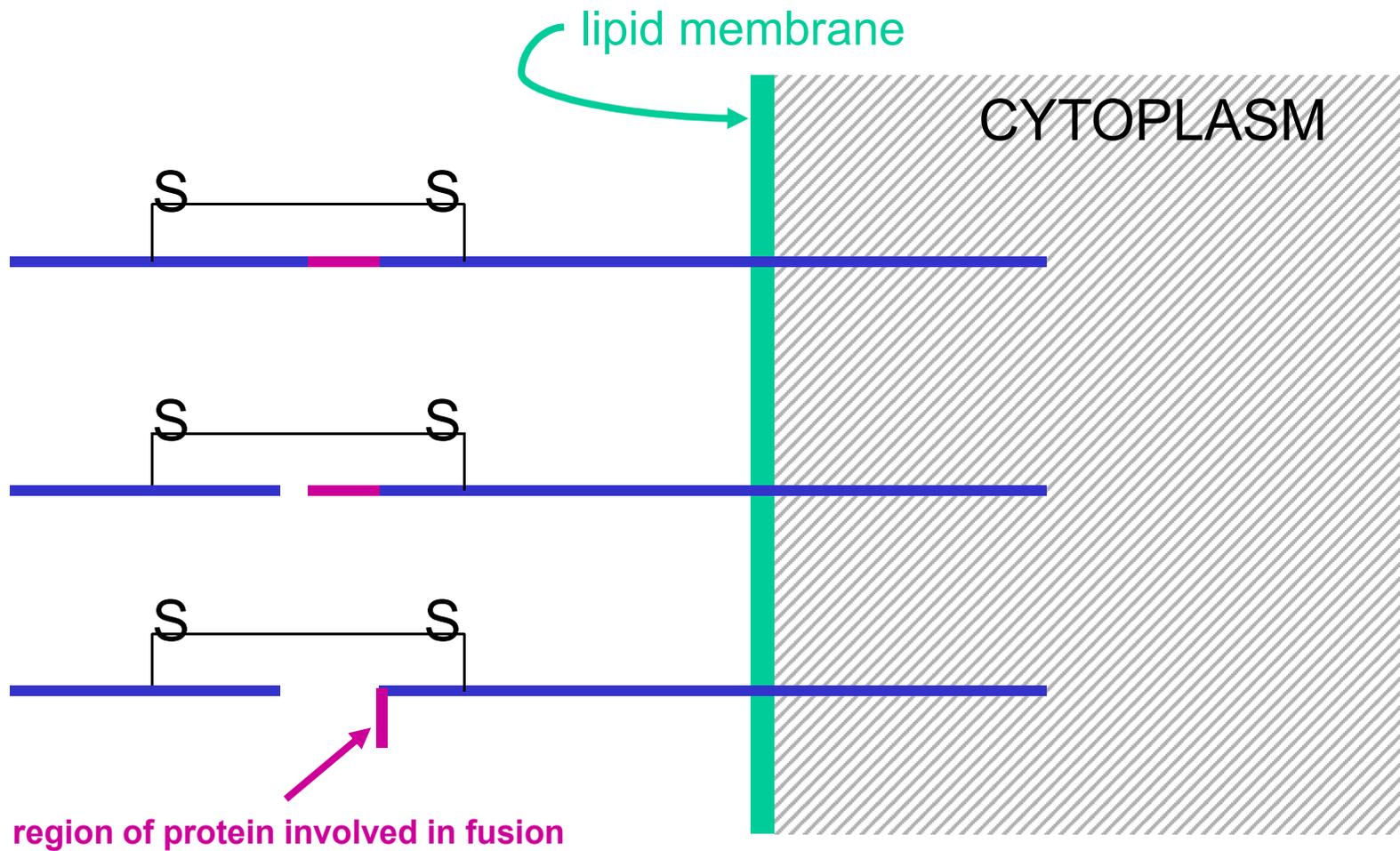
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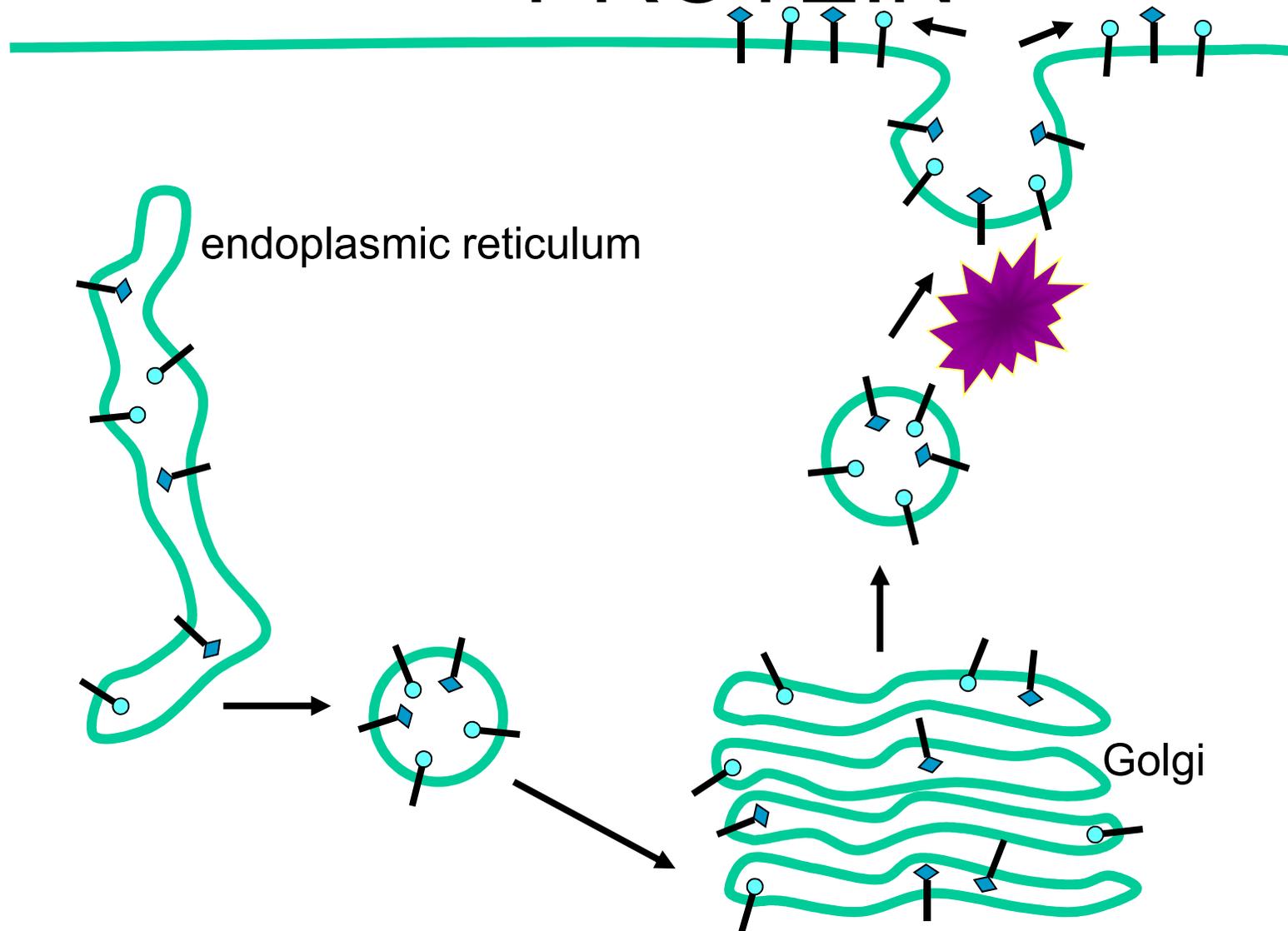
# ROLE OF NEURAMINIDASE



# ACTIVATION OF THE F PROTEIN



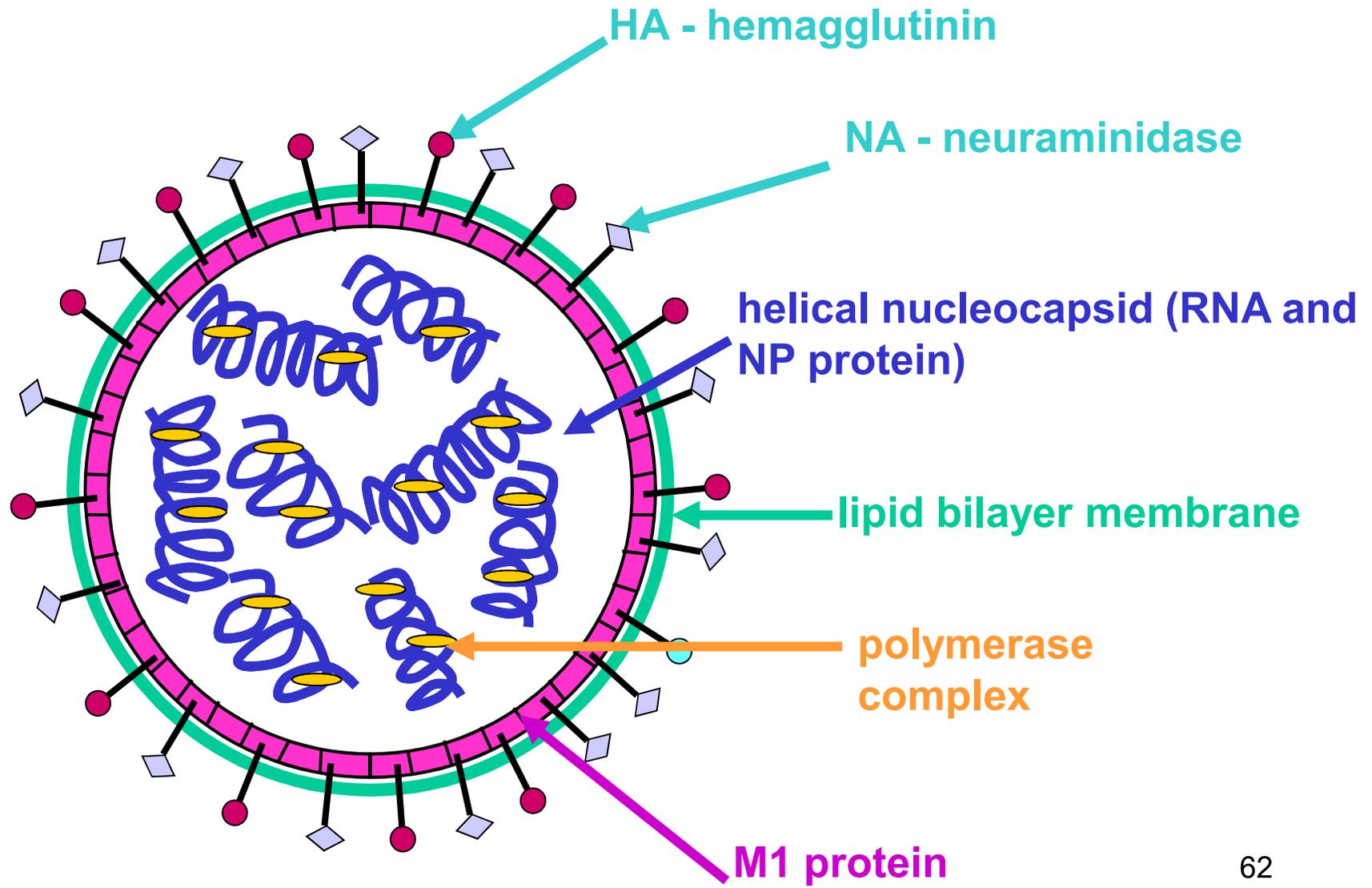
# ACTIVATION OF THE F PROTEIN



# Some differences between rhabdoviruses and paramyxoviruses

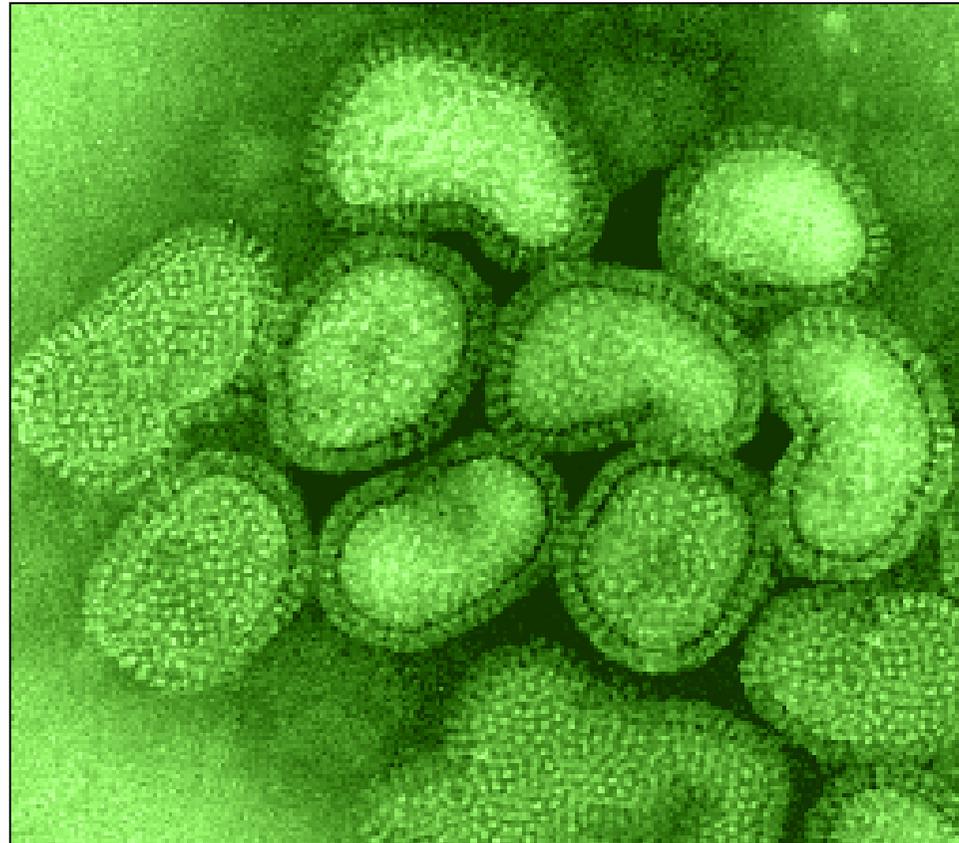
	<b>RHABDOVIRUSES</b>	<b>PARAMYXOVIRUSES</b>
shape	bullet bacilliform	round pleomorphic
glycoproteins	one (has both attachment and fusion activities)	two: one attachment one fusion
fusion pH	acidic	neutral physiological

# ORTHOMYXOVIRUSES

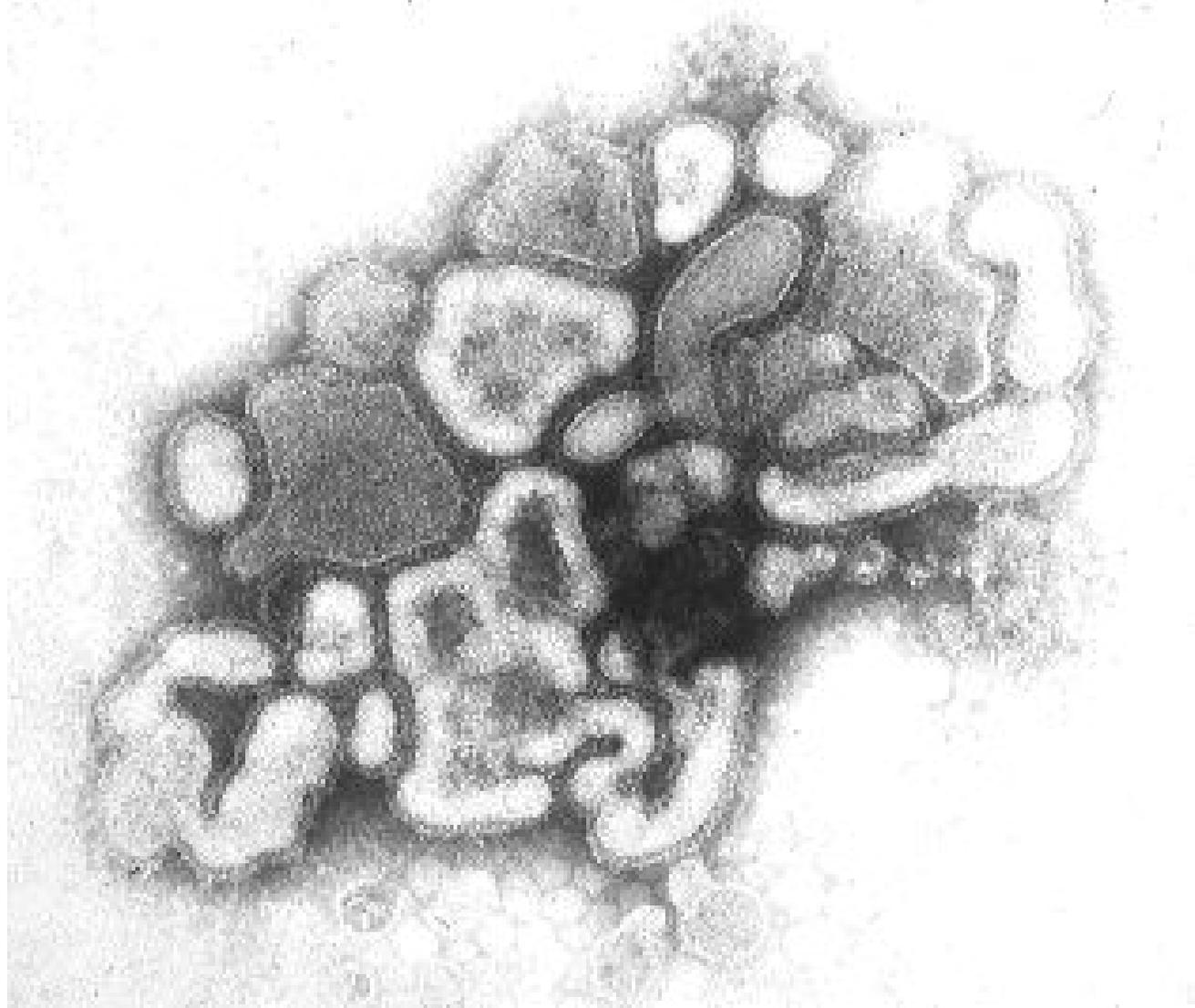


# ORTHOMYXOVIRUSES

- pleomorphic



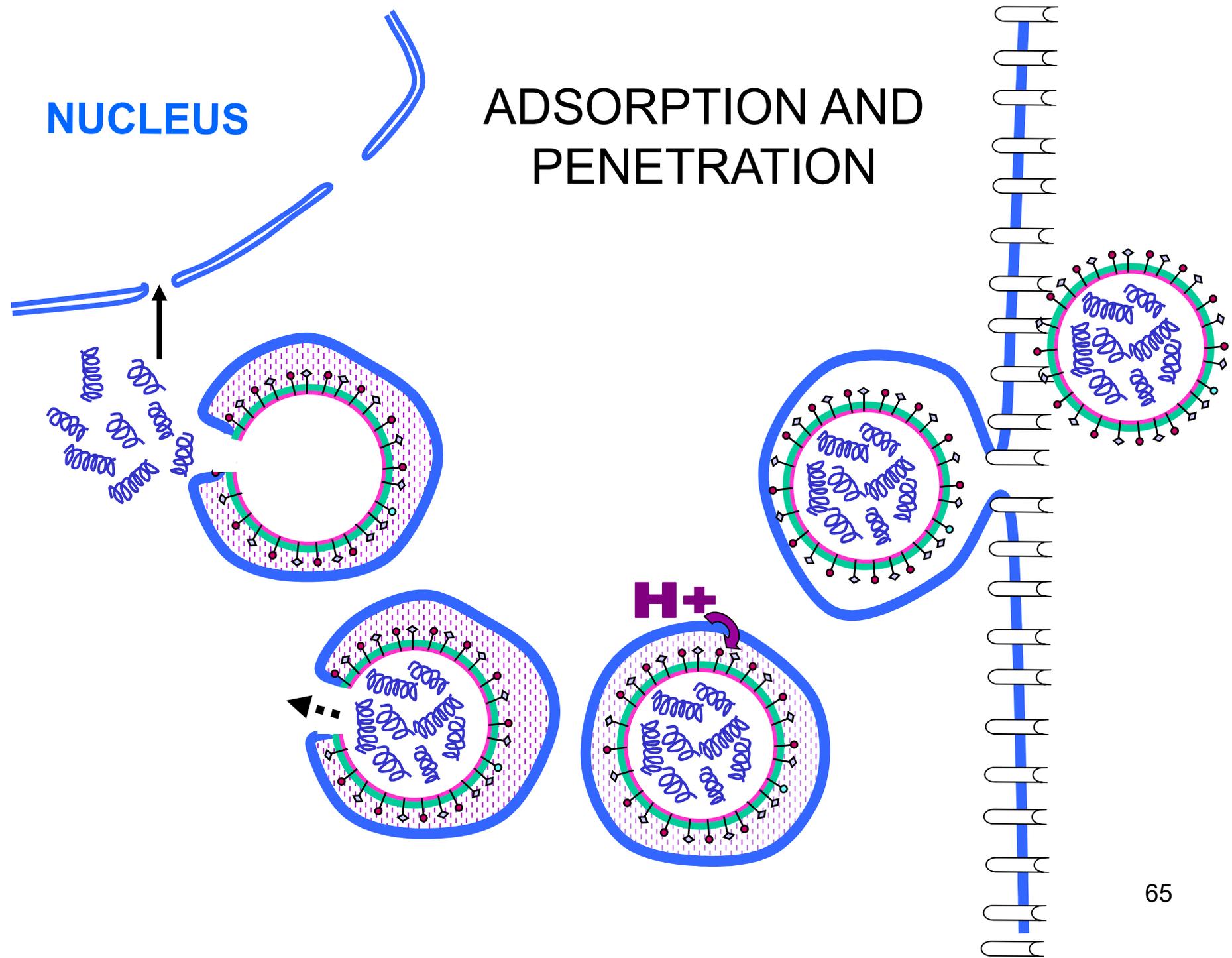
**pleomorphic**



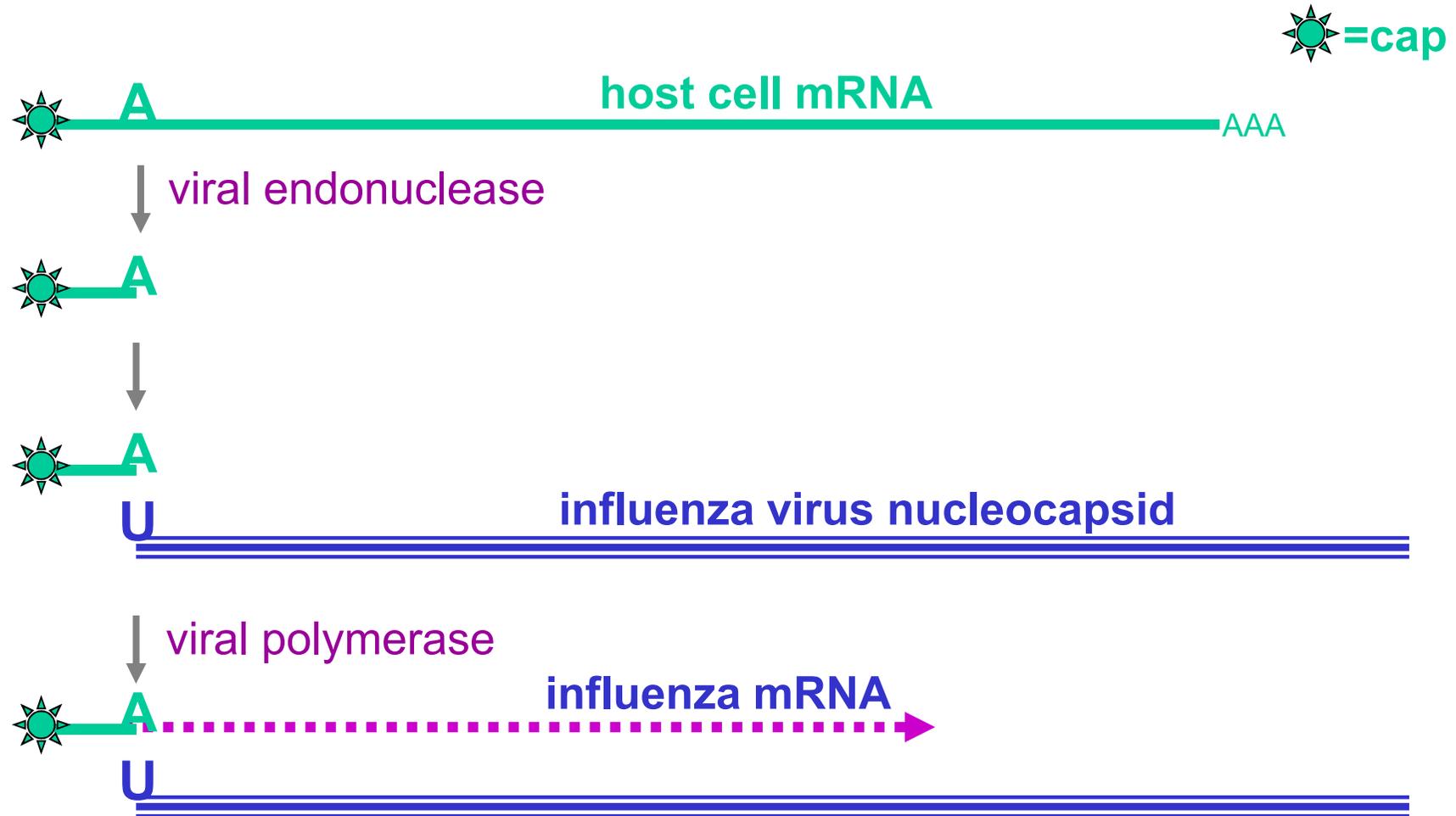
swine flu virus - CDC

NUCLEUS

# ADSORPTION AND PENETRATION



# TRANSCRIPTION (mRNA SYNTHESIS)



# RNA SYNTHESIS

- Endonuclease is virally coded and in the virion
- RNA polymerase is virally coded and in the virion
- Poly(A) polymerase is virally coded and in the virion
- Why doesn't the virus use host cell enzymes?

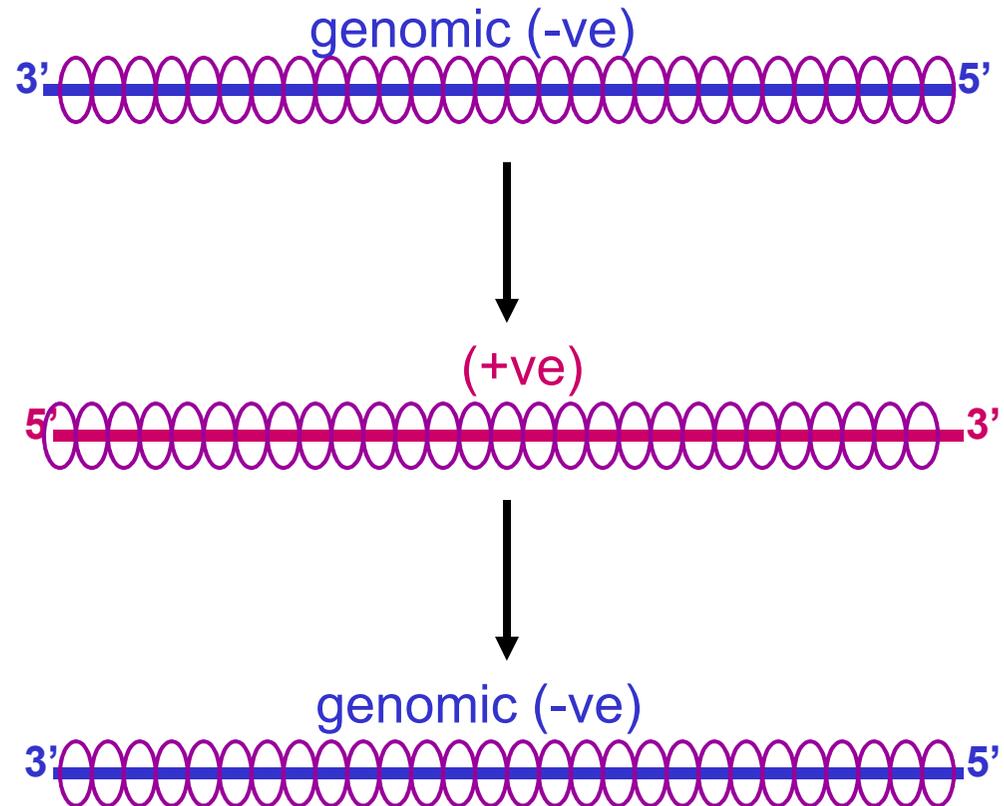
# RNA SPLICING

SEVERAL SEGMENTS GIVE RISE TO TRANSCRIPTS WHICH CAN HAVE ONE OF TWO FATES



# RNA REPLICATION

- nuclear
- authentic copies
- replicase and transcriptase probably same enzyme
- coated with protein as made
- no clear early/late



# ASSEMBLY

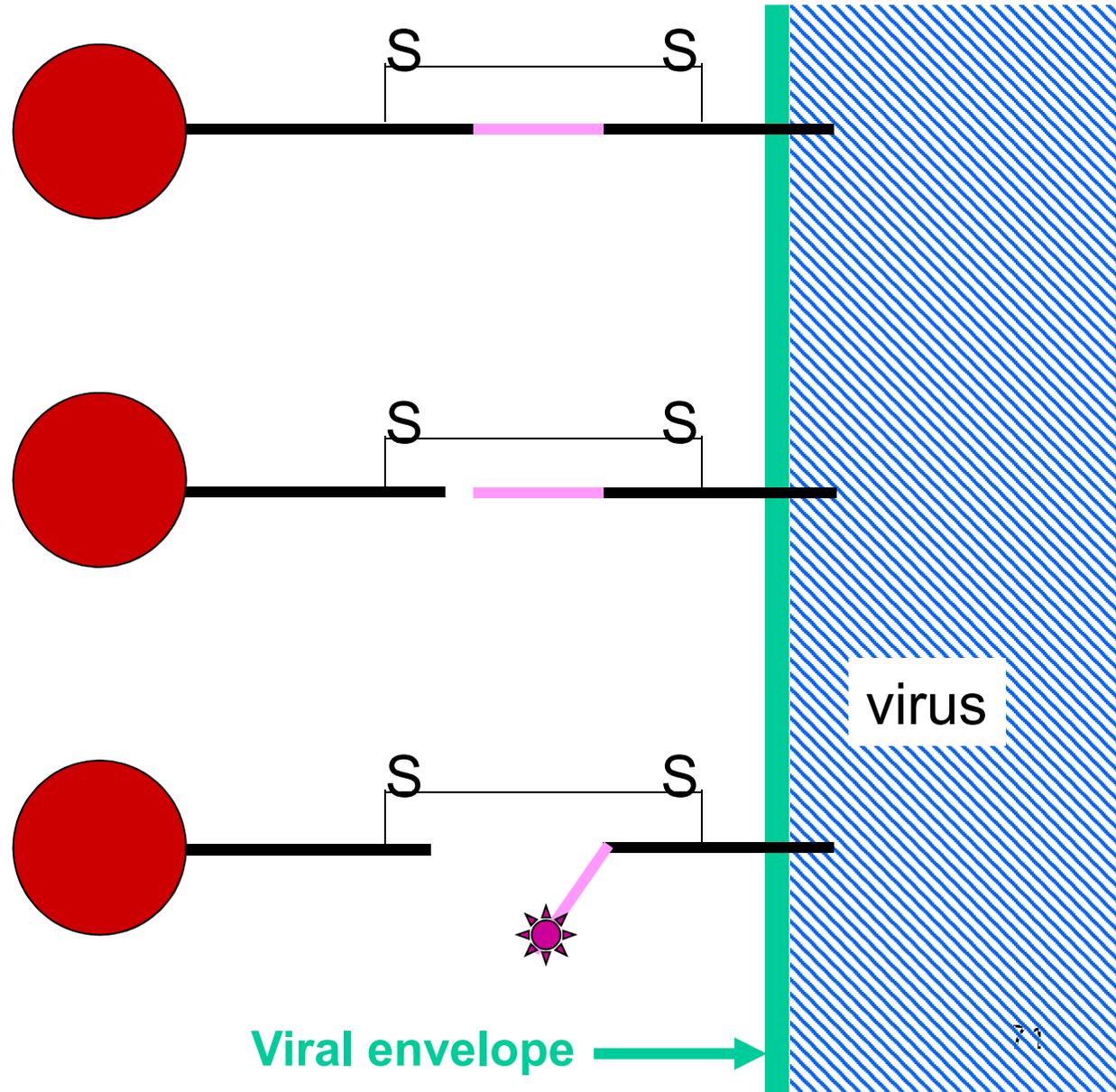
- NUCLEOCAPSIDS TRANSPORTED TO CYTOPLASM
- GLYCOPROTEINS INSERTED IN PLASMA MEMBRANE
- M PROTEINS ASSOCIATE WITH MEMBRANE
- BUD THROUGH PLASMA MEMBRANE
- HA CLEAVAGE
- ROLE OF NA

# flu HA protein and fusion

cleavage by  
host cell  
enzymes

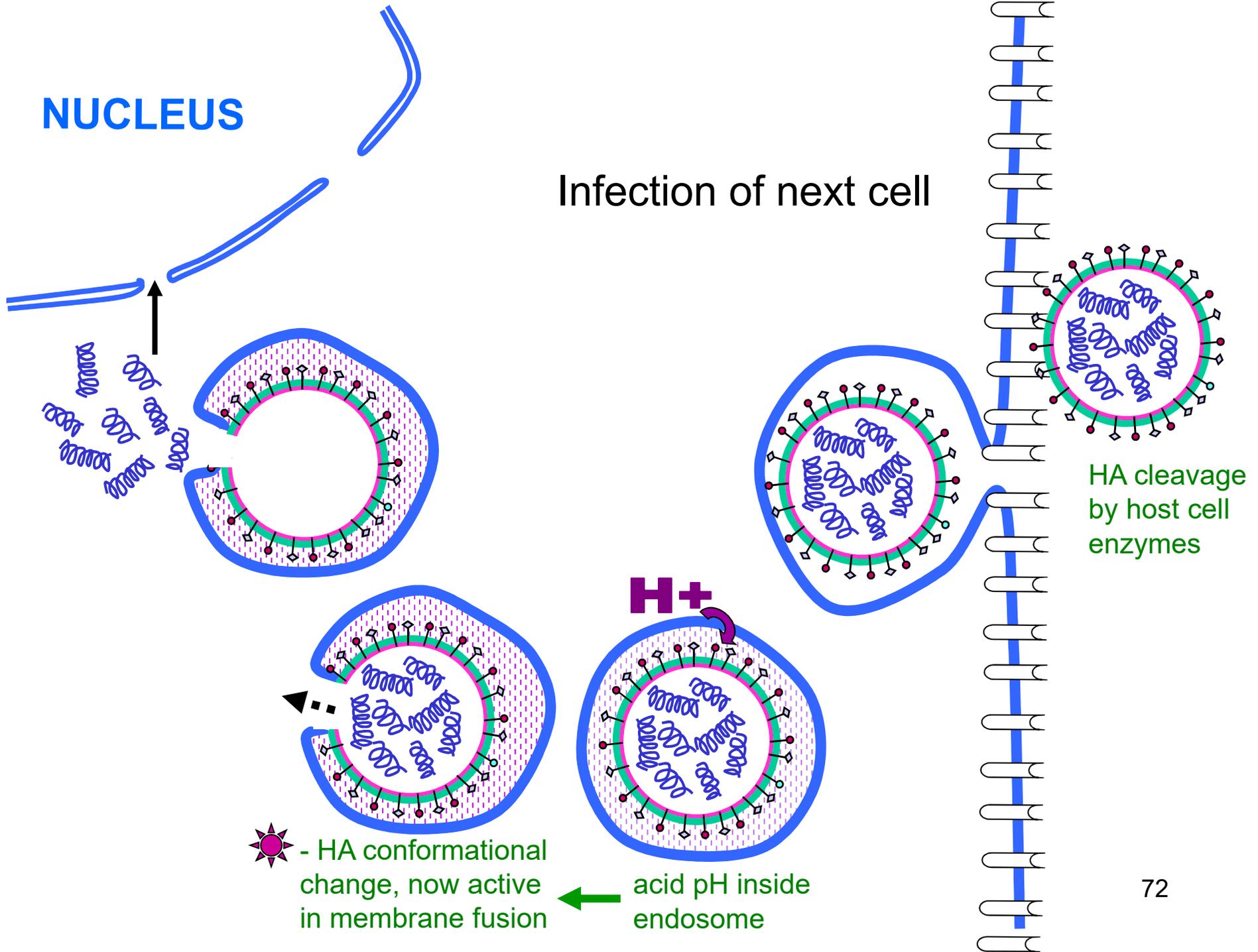
acid pH inside  
endosome

- conformational change,  
now active in membrane  
fusion



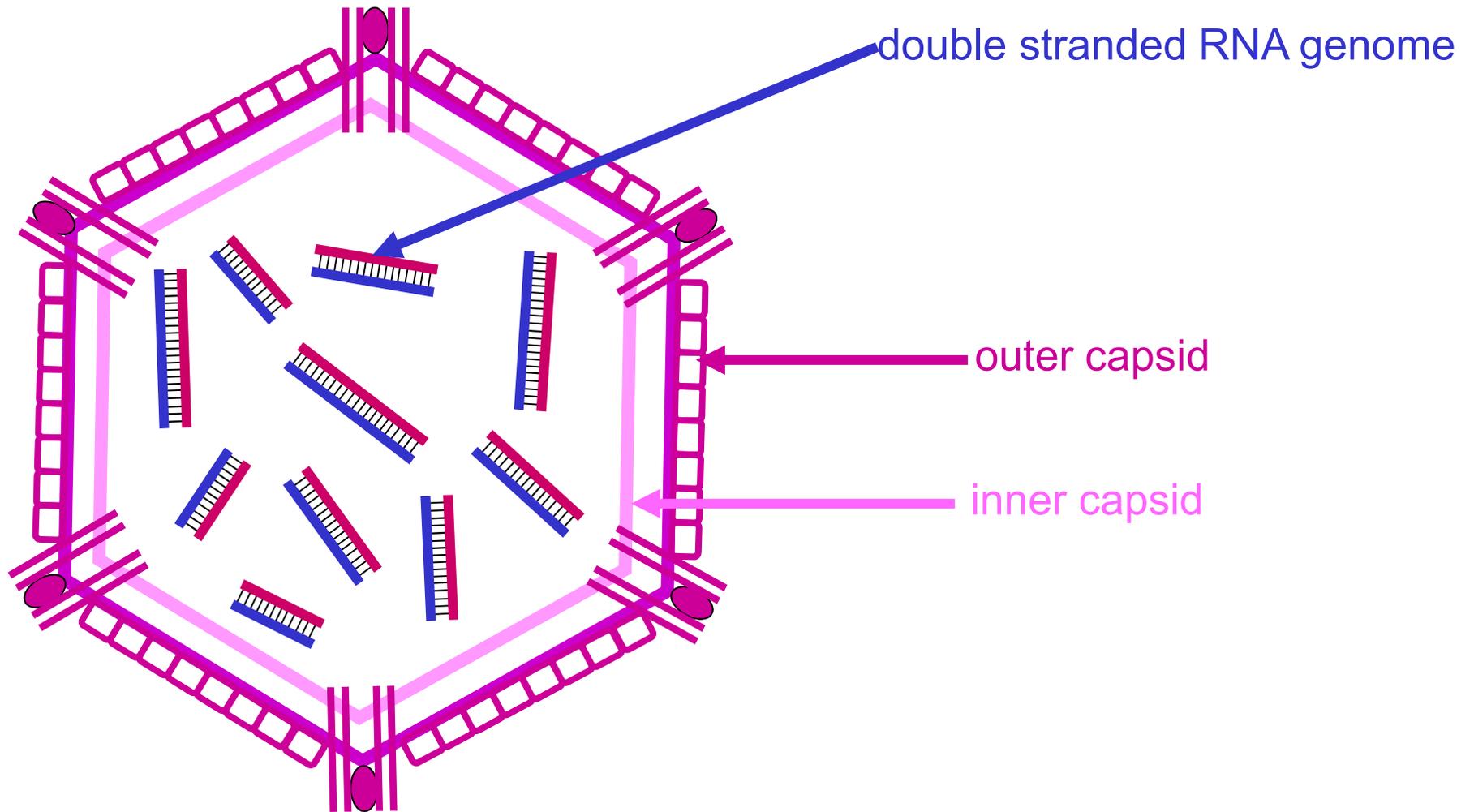
NUCLEUS

Infection of next cell



	<b>PARAMYXOVIRIDAE</b>	<b>ORTHOMYXOVIRIDAE</b>
<b>genome</b>	<b>nonsegmented</b>	<b>segmented</b>
<b>RNA synthesis</b>	<b>cytoplasmic</b>	<b>nuclear</b>
<b>need for RNA primer</b>	<b>no</b>	<b>yes</b>
<b>hemagglutinin, neuraminidase</b>	<b>if both, part of same protein (HN)</b>	<b>Influenza A and B viruses have both, but on different proteins (HA and NA)</b>
<b>syncytial formation</b>	<b>yes (F functions at physiol. pH)</b>	<b>no (HA functions at acid pH)</b>

# REOVIRUS FAMILY

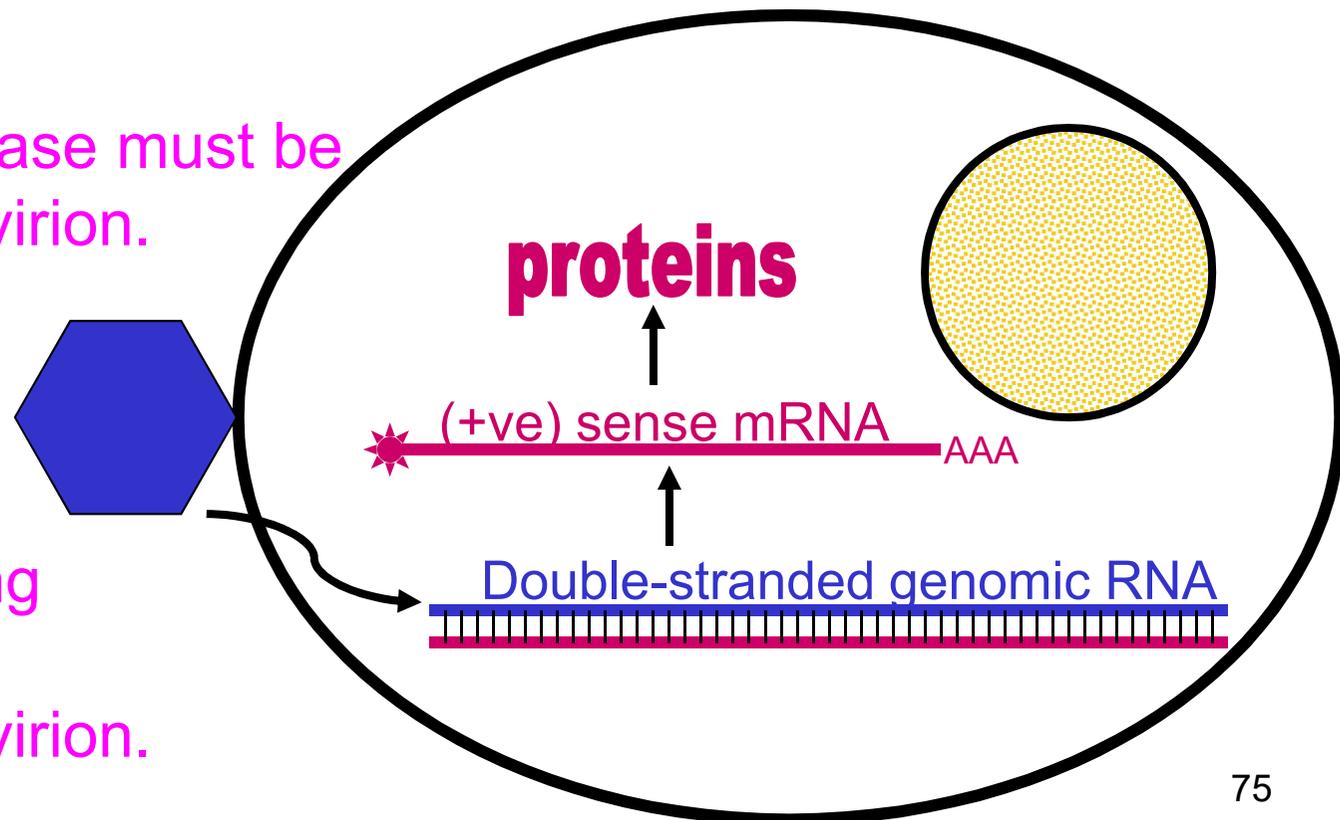


# Need to make mRNA

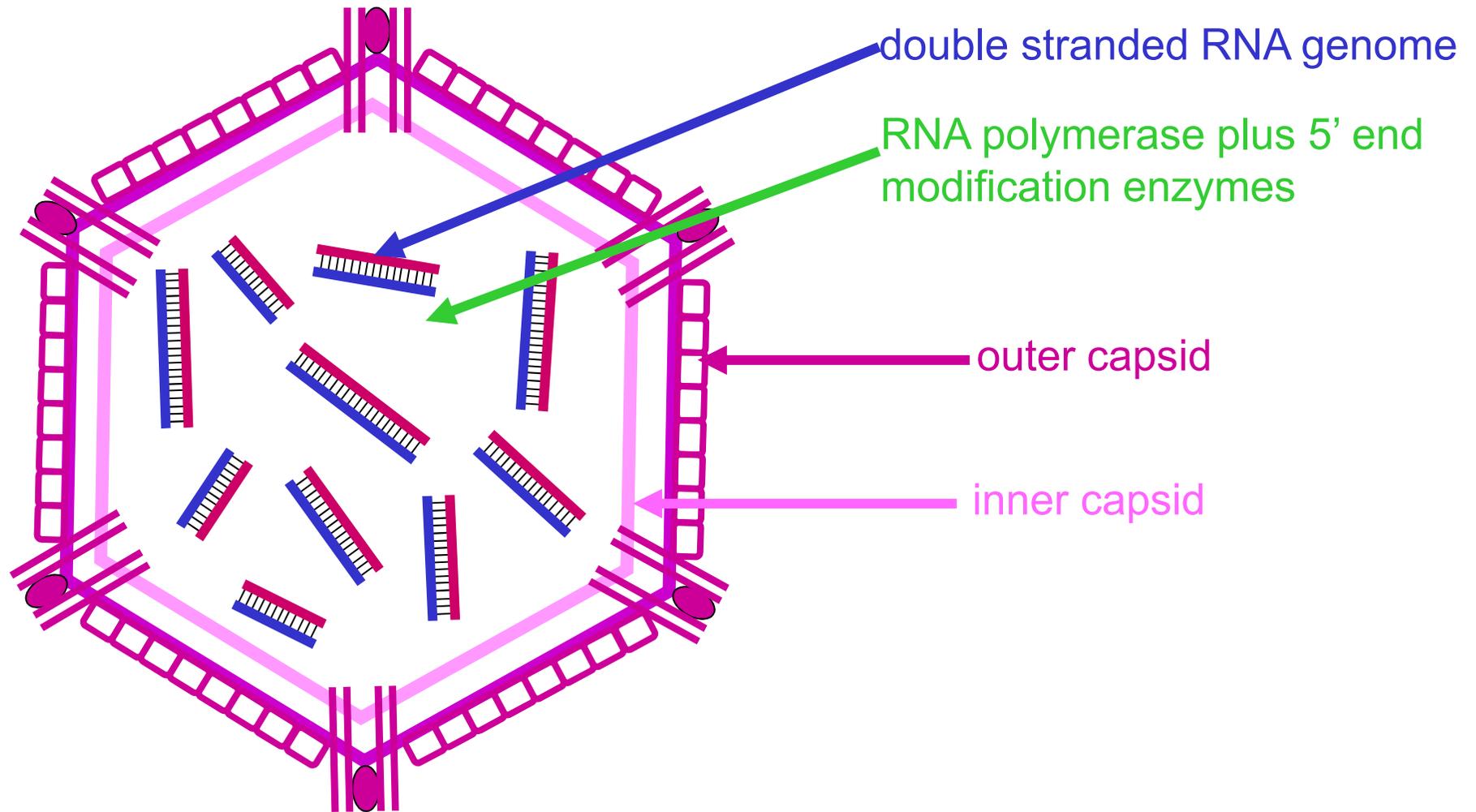
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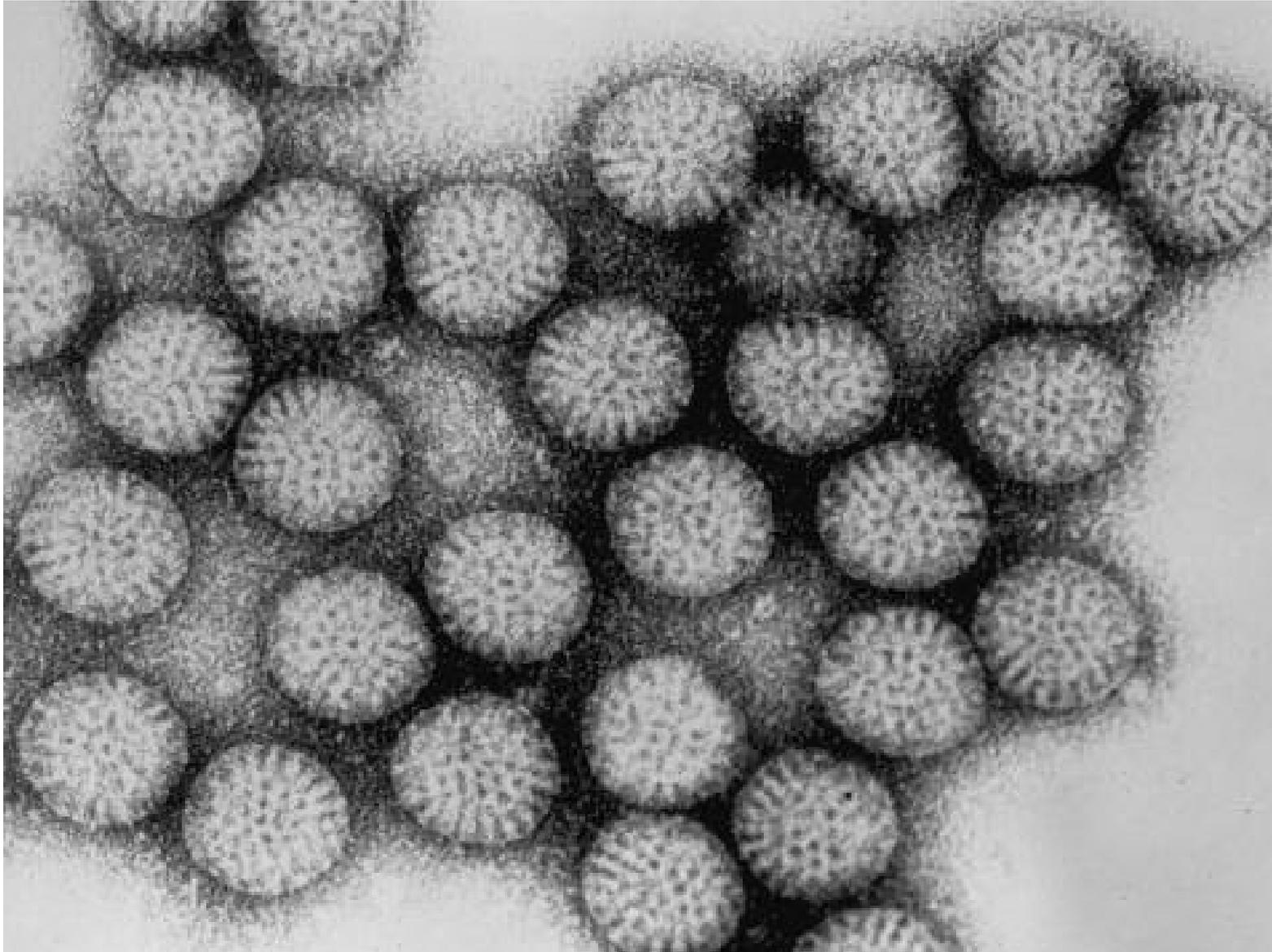
RNA modifying enzymes are packaged in virion.



# REOVIRUS FAMILY



# ROTAVIRUS

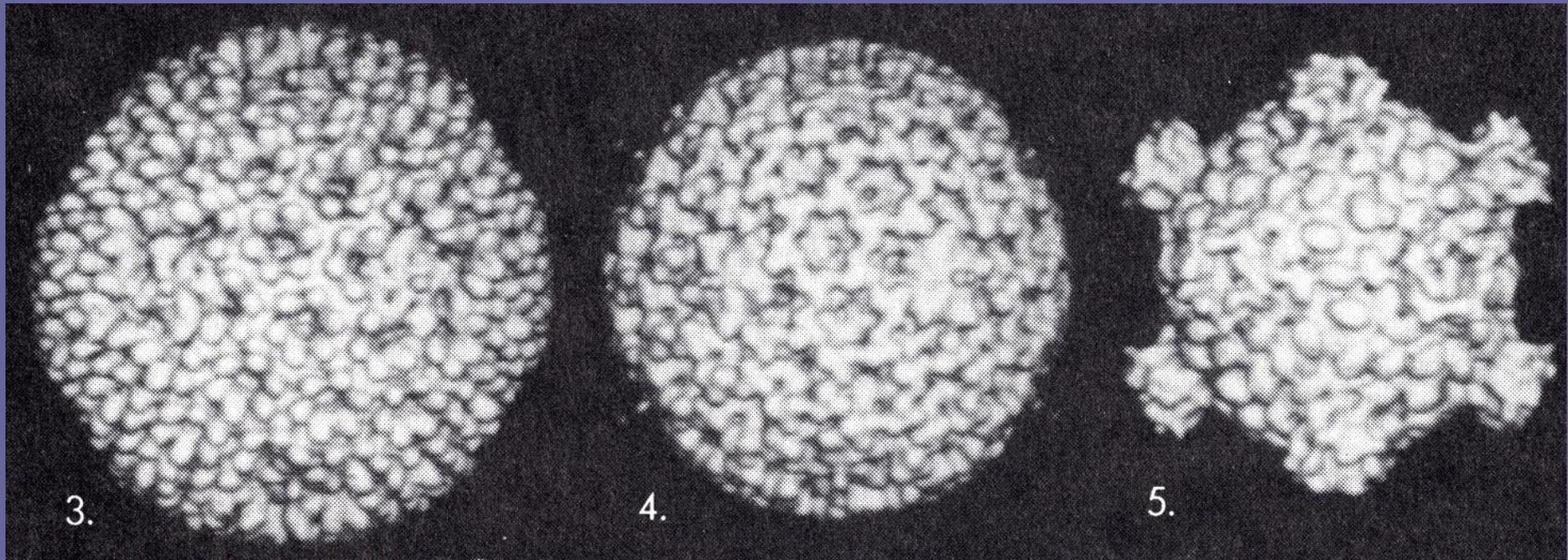


Erskine Palmer, CDC

# REOVIRUS FAMILY

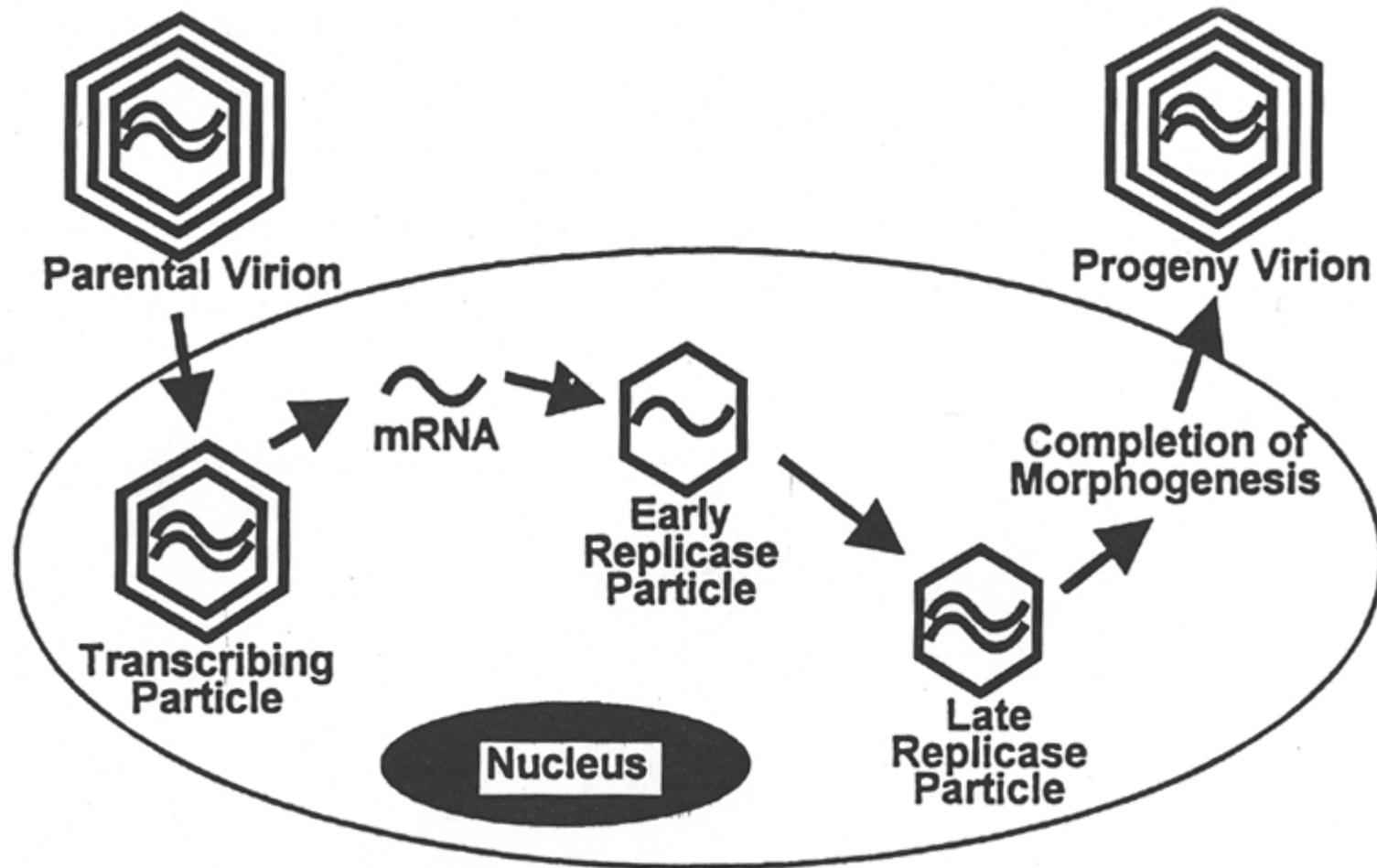
- INCLUDES
  - REOVIRUSES
  - ROTAVIRUSES
    - IMPORTANT HUMAN PATHOGENS
  - ORBIVIRUSES
    - INCLUDE COLORADO TICK FEVER VIRUS

# ADSORPTION, PENETRATION, UNCOATING



# ADSORPTION, PENETRATION, UNCOATING

- proteases in GI tract
- attachment proteins at vertices
- cross membrane directly or via endosomes



# RELEASE

- TRANSIENTLY ENVELOPED
  - BUD INTO ER AND GET ENVELOPED
  - MEMBRANE IS LOST
  - RELEASED BY LYSIS