



Orthomyxoviruses

Defense and Barriers
January 25, 2024

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Why I love studying influenza:

1. Influenza is virologically interesting!
2. Influenza evolves rapidly, and jumps between host species frequently, making it evolutionarily exciting
3. Influenza has enormous impacts on public health for wild animals, domestic animals (companion, and agriculture), and humans

Learning objectives

1. Describe the structure of influenza A (orthomyxovirus) virus.
2. Draw the Influenza replication cycle including the role of hemagglutinin, neuraminidase, matrix protein (M1), Ion channels M2, cap-snatching.
3. Define antigenic shift and antigenic drift in the context of Influenza and how these may lead to seasonal variation and endemicity.
4. Describe why birds and pigs are critical in global epidemiology of Influenza.
5. Differentiate between LPAI and HPAI.

Outline:

1. Influenza structure and life cycle
2. Influenza host ecology, evolution, pandemic virus formation, and antigenic drift
3. Emerging public health issues in influenza
 - Global spread of high path avian influenza
 - Canine and equine influenzas

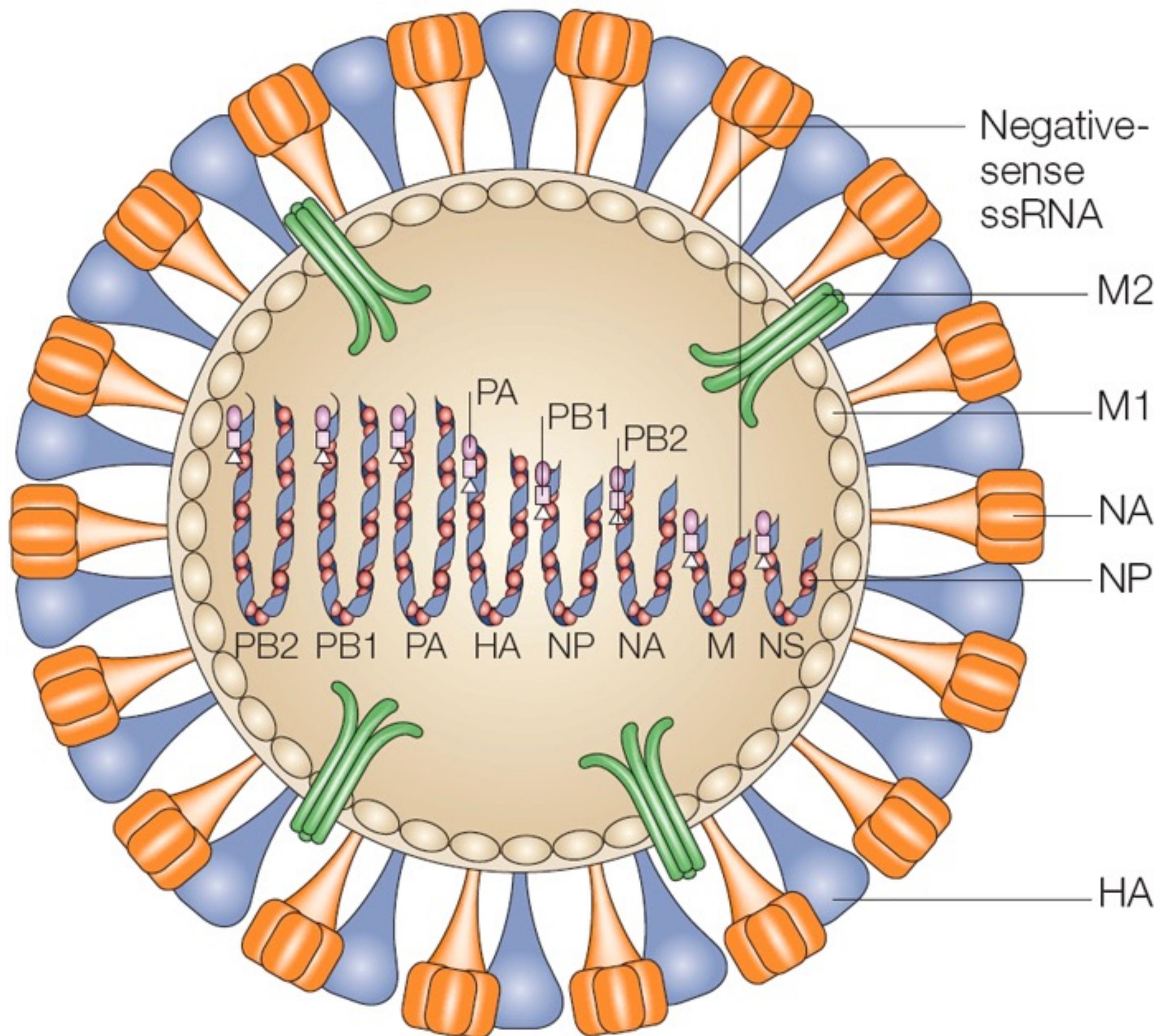
Outline:

- 1. Influenza structure and life cycle**
2. Influenza host ecology, evolution, and pandemic virus formation
3. Emerging public health issues in influenza
 - Global spread of high path avian influenza
 - Canine and equine influenzas
 - Vaccination as a control strategy for influenza, and antigenic drift

Orthomyxoviruses are negative sense, single stranded RNA viruses

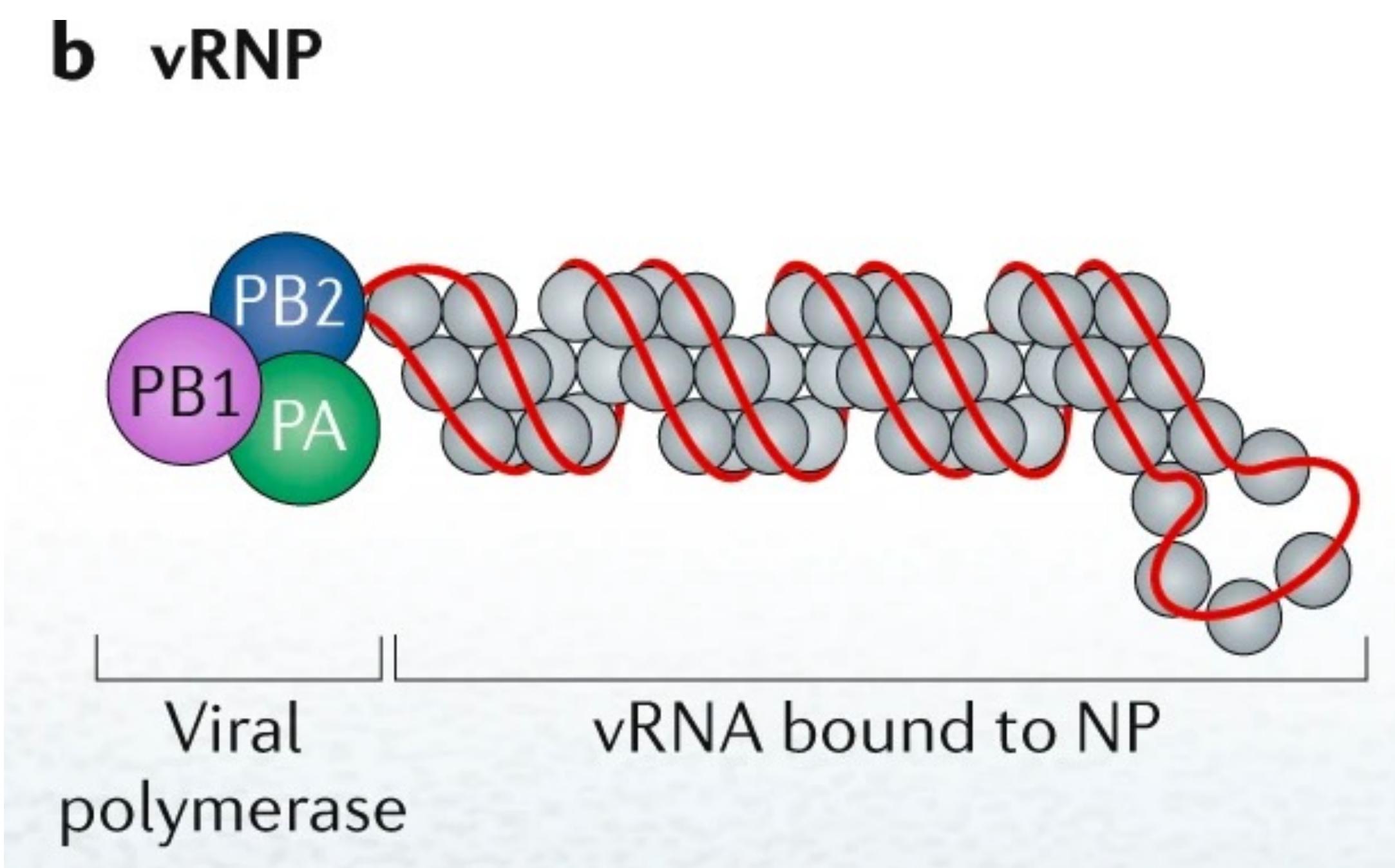
- 1. Alphainfluenza viruses (influenza A): birds to humans and other mammals**
- 2. Betainfluenza viruses (influenza B): humans to seals**
- 3. Gammainfluenzaviruses (influenza C): humans to pigs**
- 4. Deltainfluenzaviruses (influenza D): cows to pigs, humans, camels, sheep**
- 5. Isavirus, Thogotovirus, Quaranjavirus**

Influenza virion structure



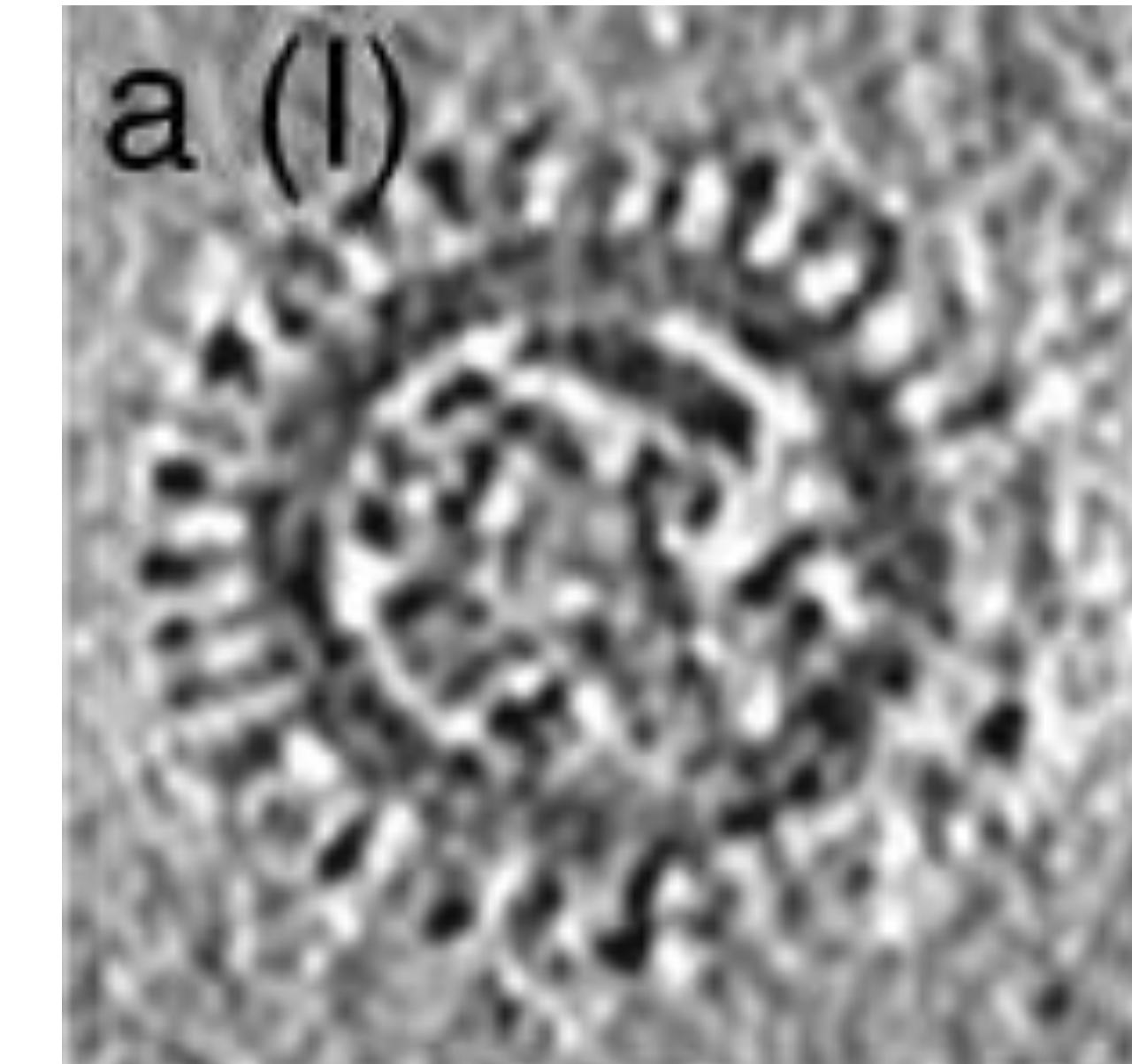
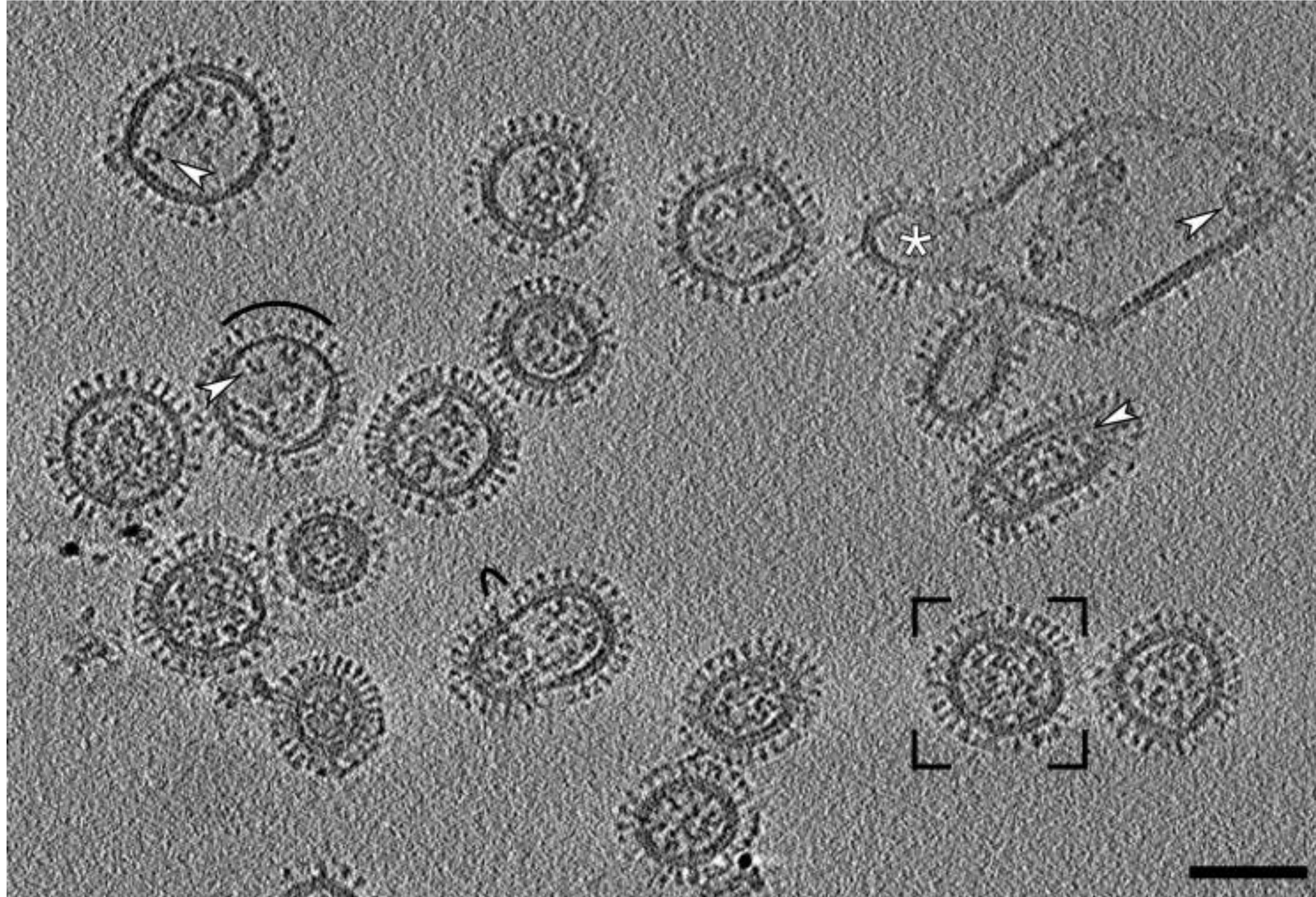
1. Single stranded, negative sense, segmented RNA genome with 8 gene segments
2. Contains a capsid made of M1 protein
3. Encapsidated with a host-derived envelope
4. 2 surface glycoproteins are embedded in envelope, Hemagglutinin (HA) and neuraminidase (NA), and an ion channel (M2)
5. Influenza viruses are subtyped by the HA and NA types (e.g., H1N1, H3N2, etc..)

The influenza genome is packaged as a viral ribonucleoprotein (vRNP)



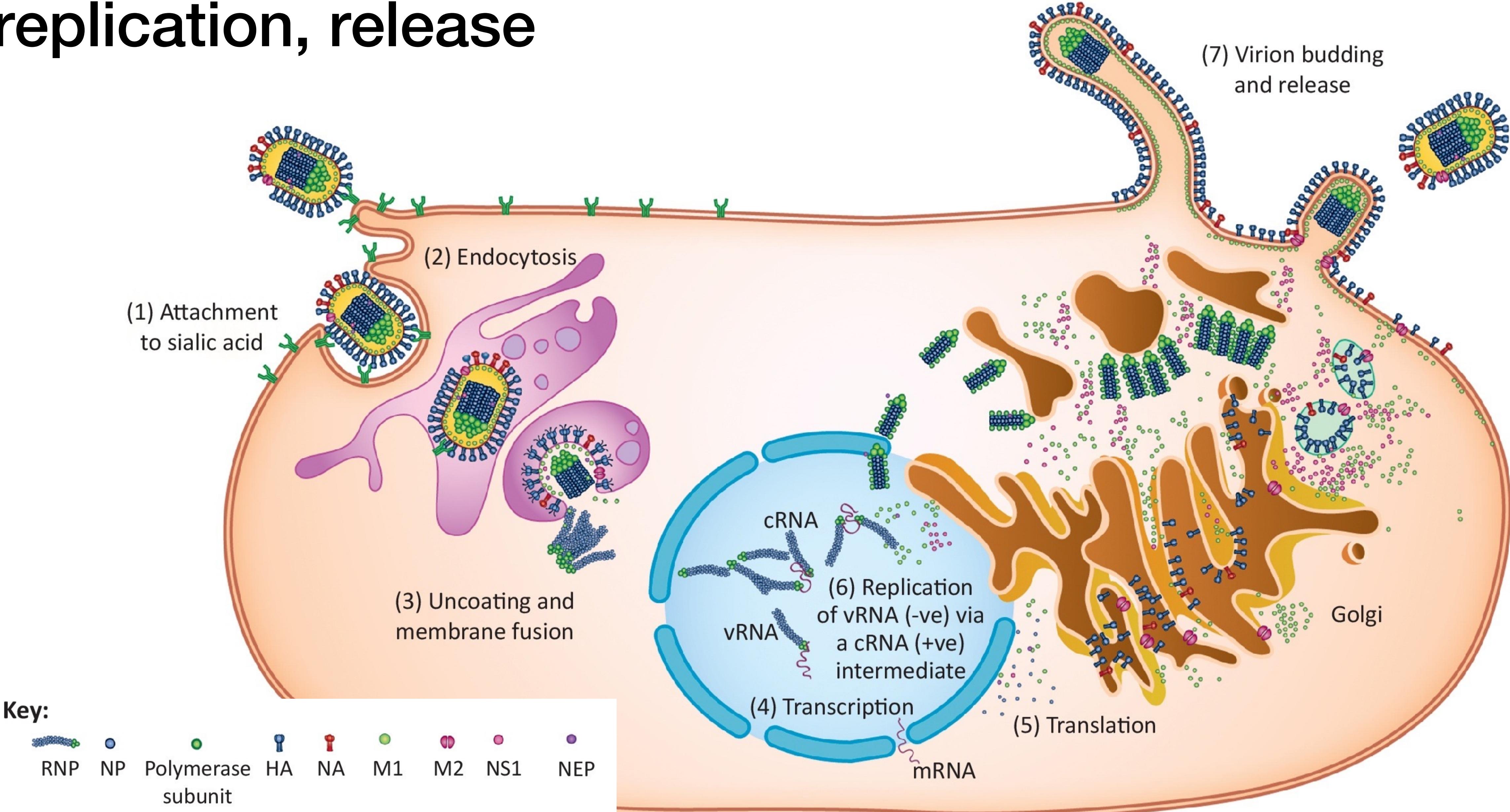
1. Each viral gene segment enters the cell bound by nucleoprotein (NP) and a polymerase complex
2. Polymerase complex is a trimeric complex comprised of PB2, PB1, PA
3. Genome packaging as a vRNP is necessary because:
 - Naked, negative sense RNA will trigger host immune responses. Covering vRNA with NP hides it.
 - Cellular polymerases cannot produce mRNA from negative sense RNA, so influenza must provide its own polymerase. -> viral RNA alone is not infectious

The influenza virion, as visualized via cryo-electron tomography

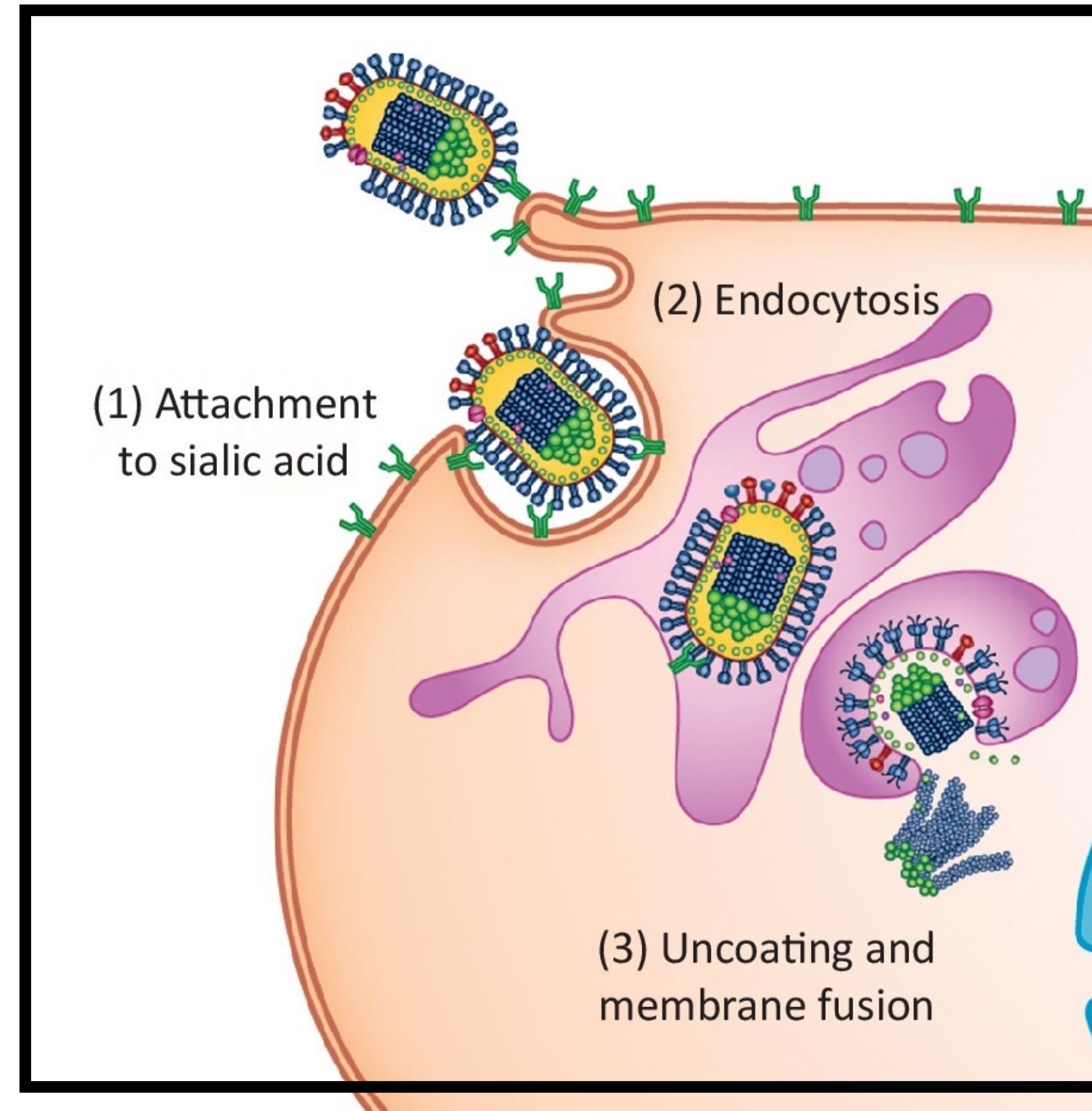


* Harris et al, PNAS 2006

The influenza lifecycle has 3 main steps: entry, replication, release

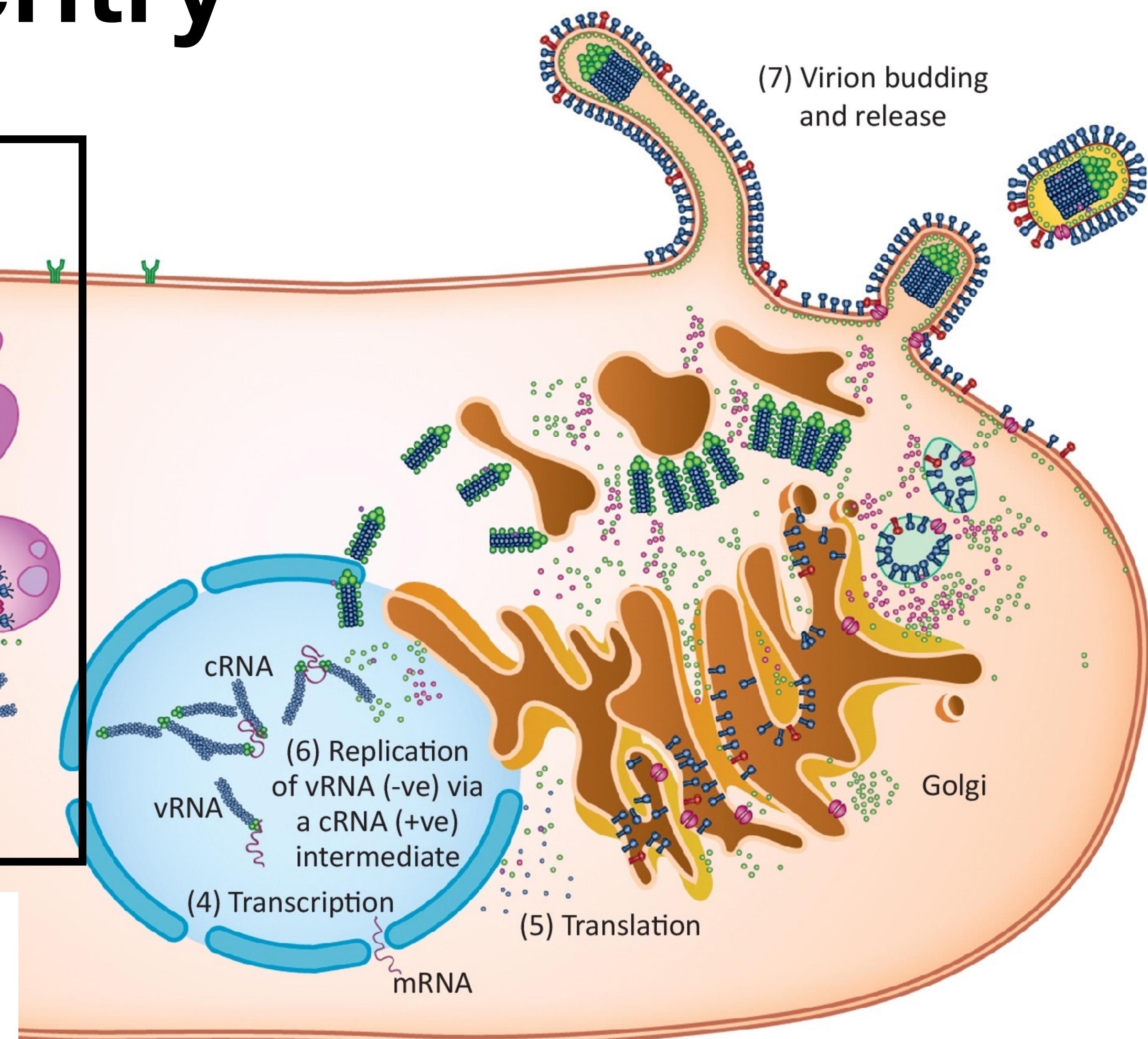


Step 1: cell entry



Key:

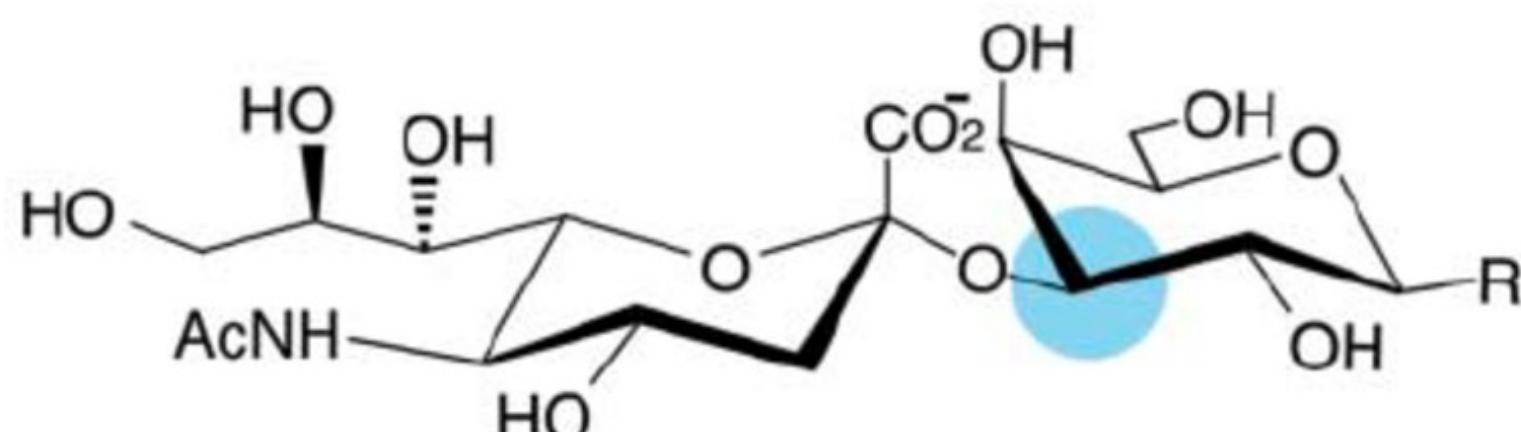
RNP	NP	Polymerase subunit	HA	NA	M1	M2	NS1	NEP
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The influenza receptor are sialic acids, which are common, terminal sugars whose type and conformation vary across host species. Receptor binding specificity (α -2,6 vs. α -2,3 binding) is a major determinant of host specificity

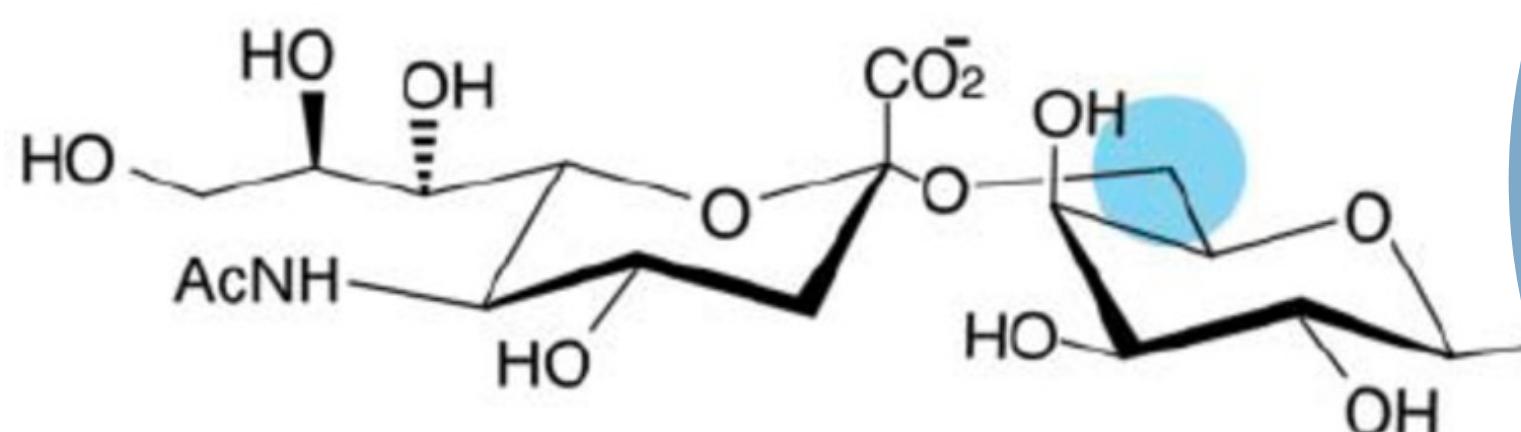
a

Neu5 $\text{Ac}\alpha$ 2-3Gal

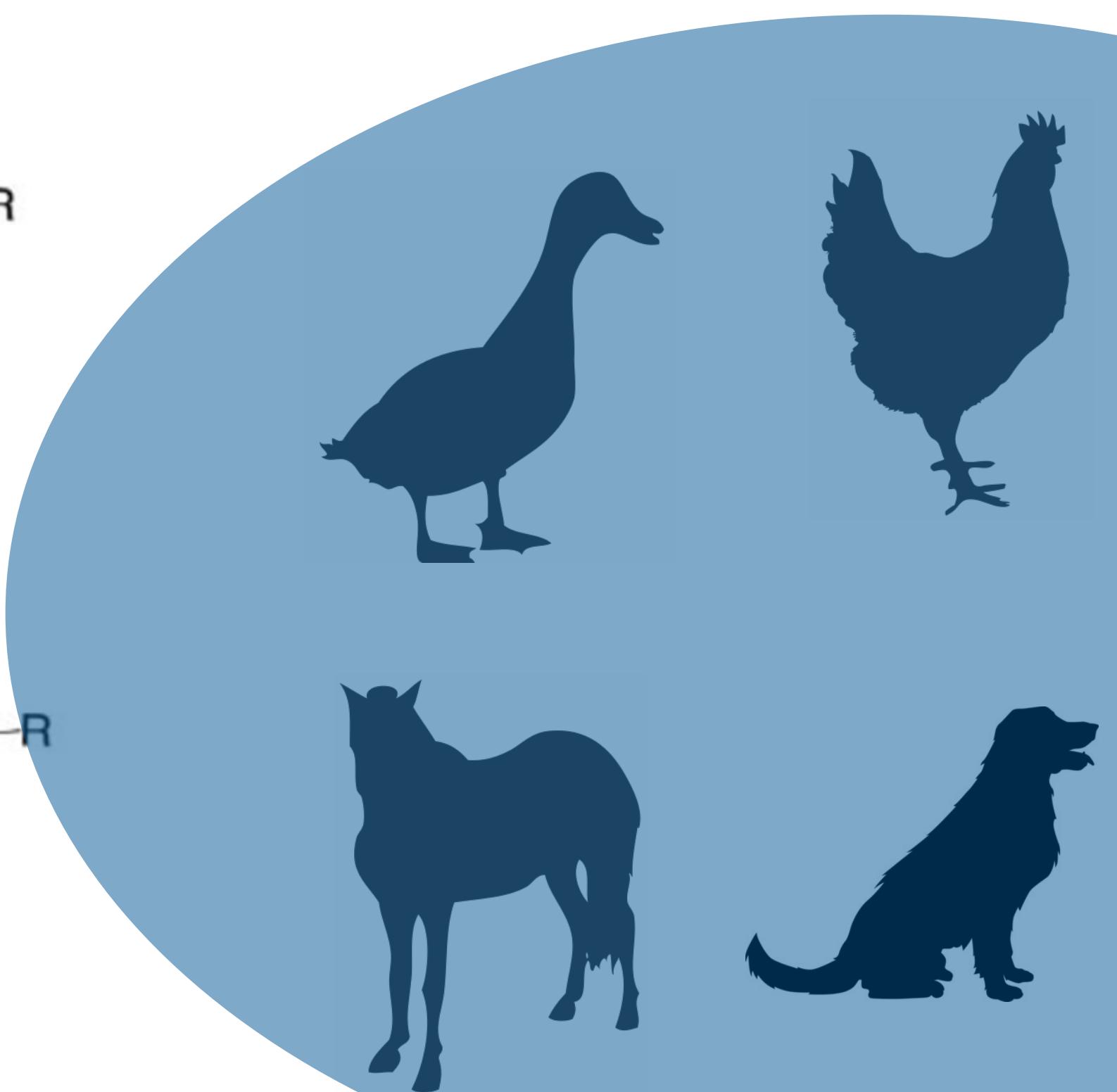


b

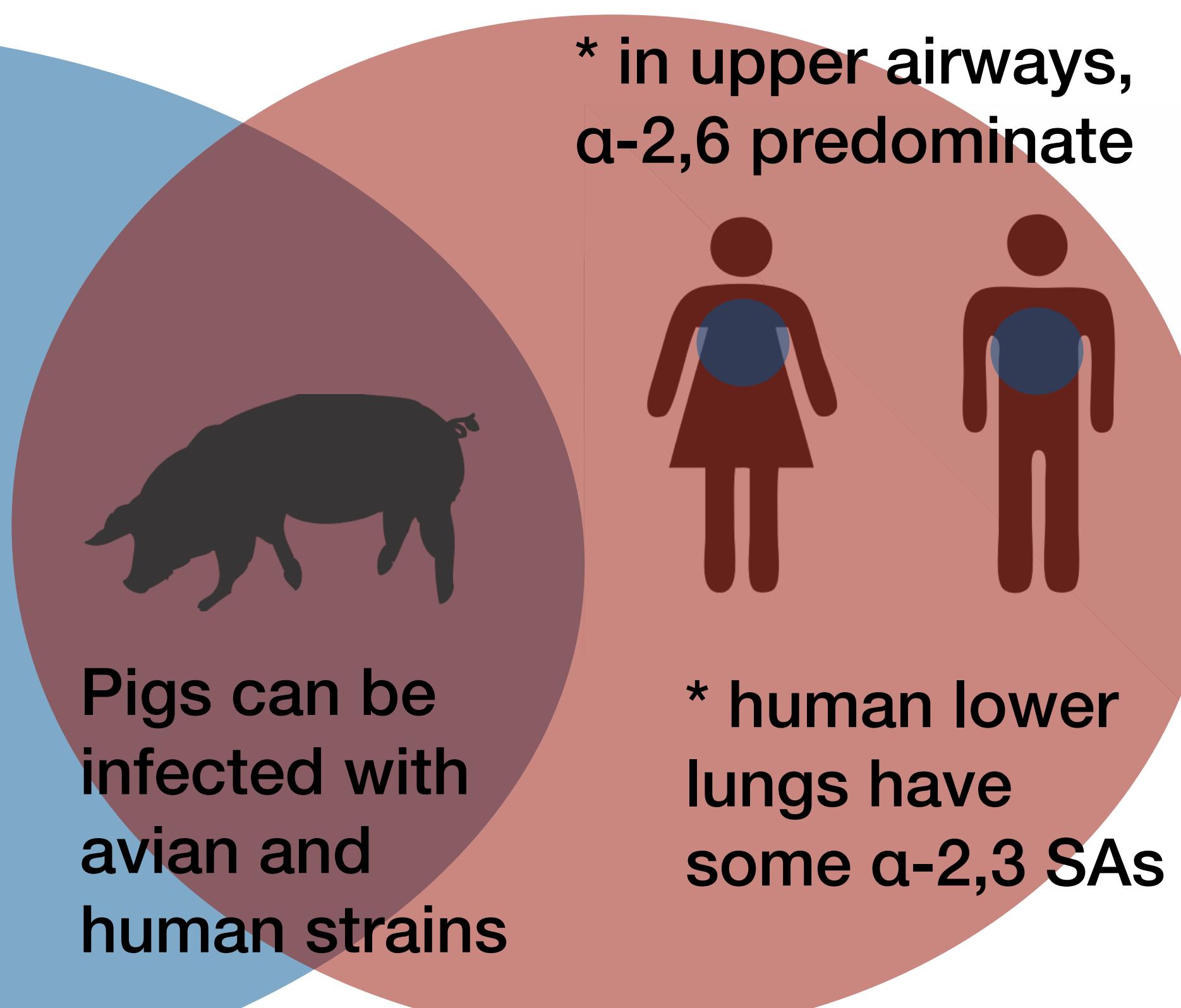
Neu5 $\text{Ac}\alpha$ 2-6Gal



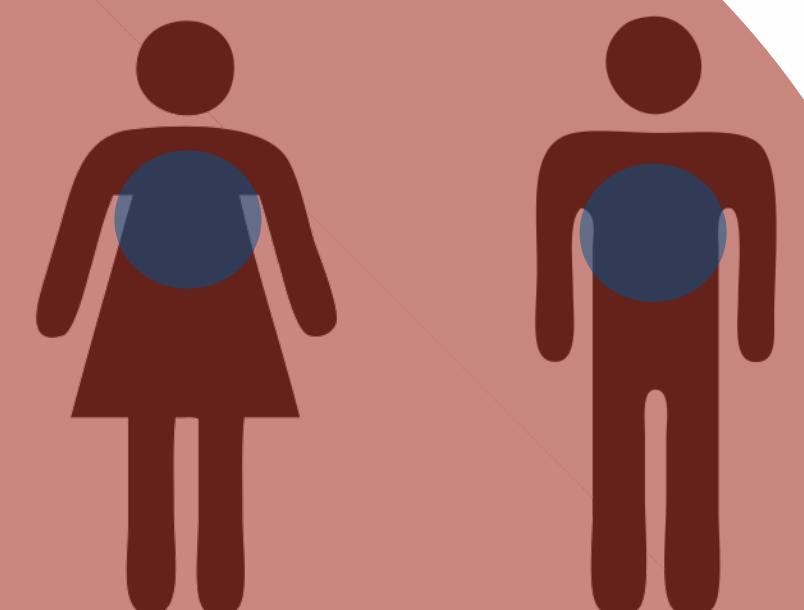
Primarily bind α -2,3
linked sialic acids



Primarily bind α -2,6
linked sialic acids

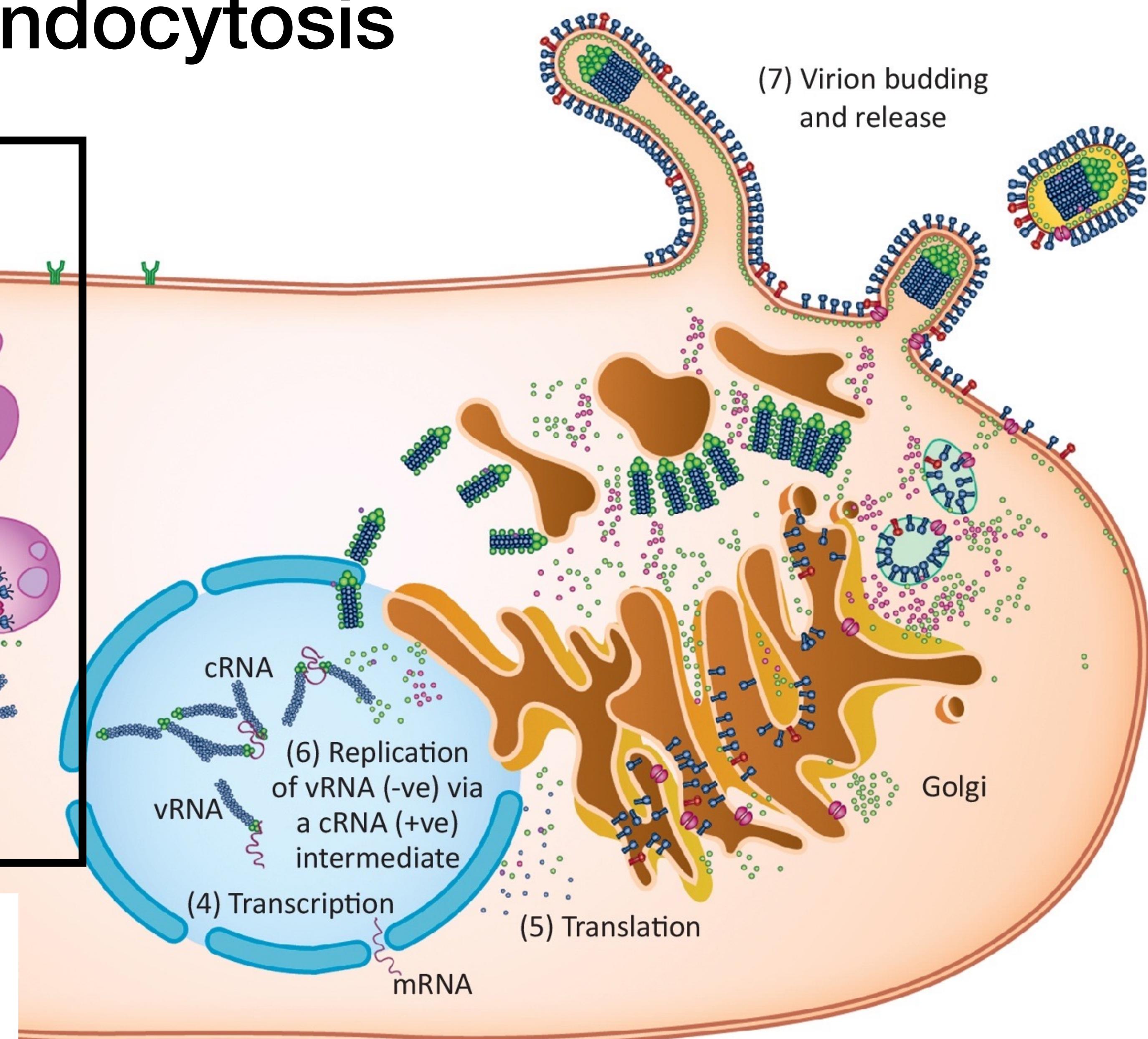
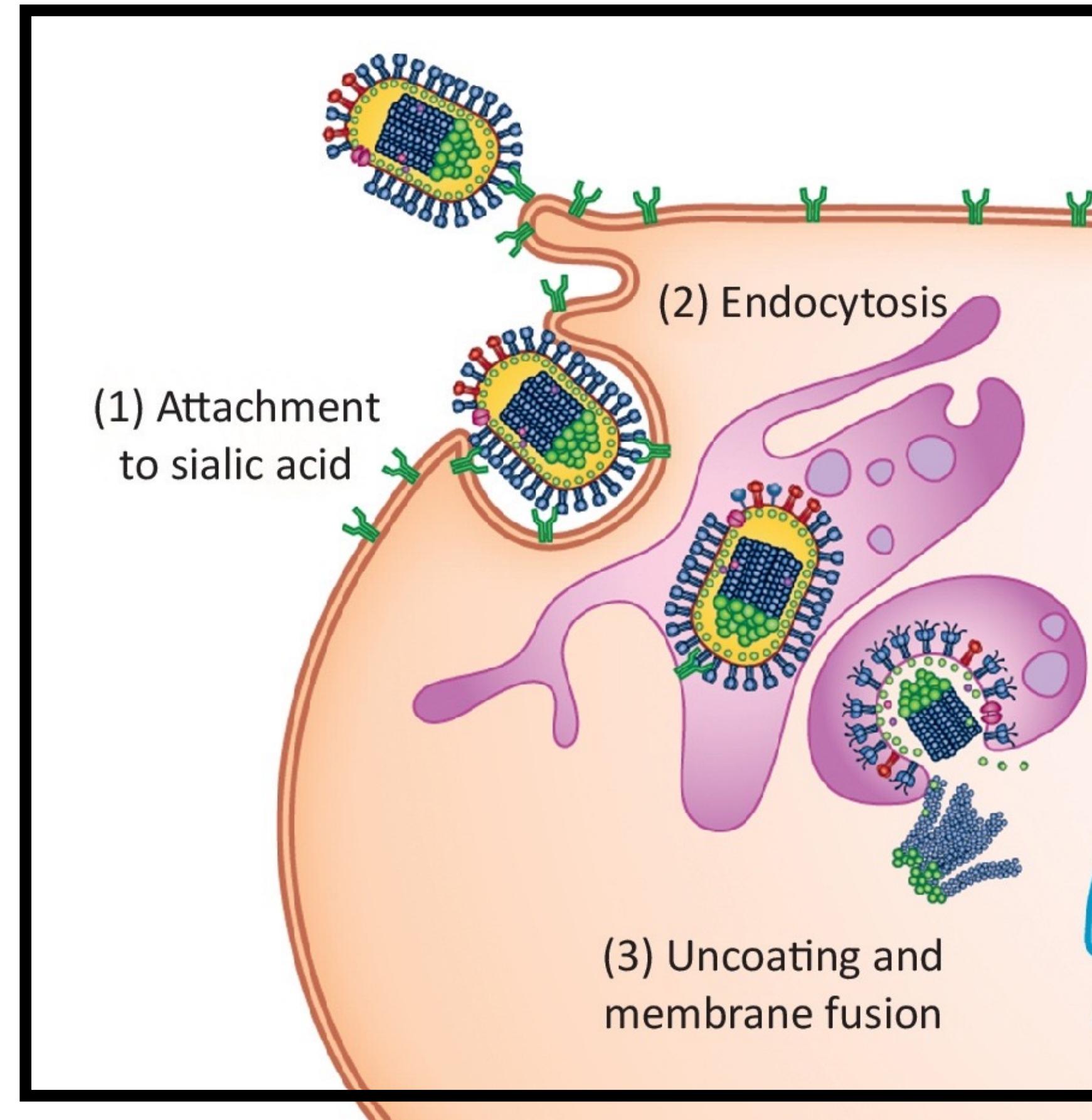


* in upper airways,
 α -2,6 predominate



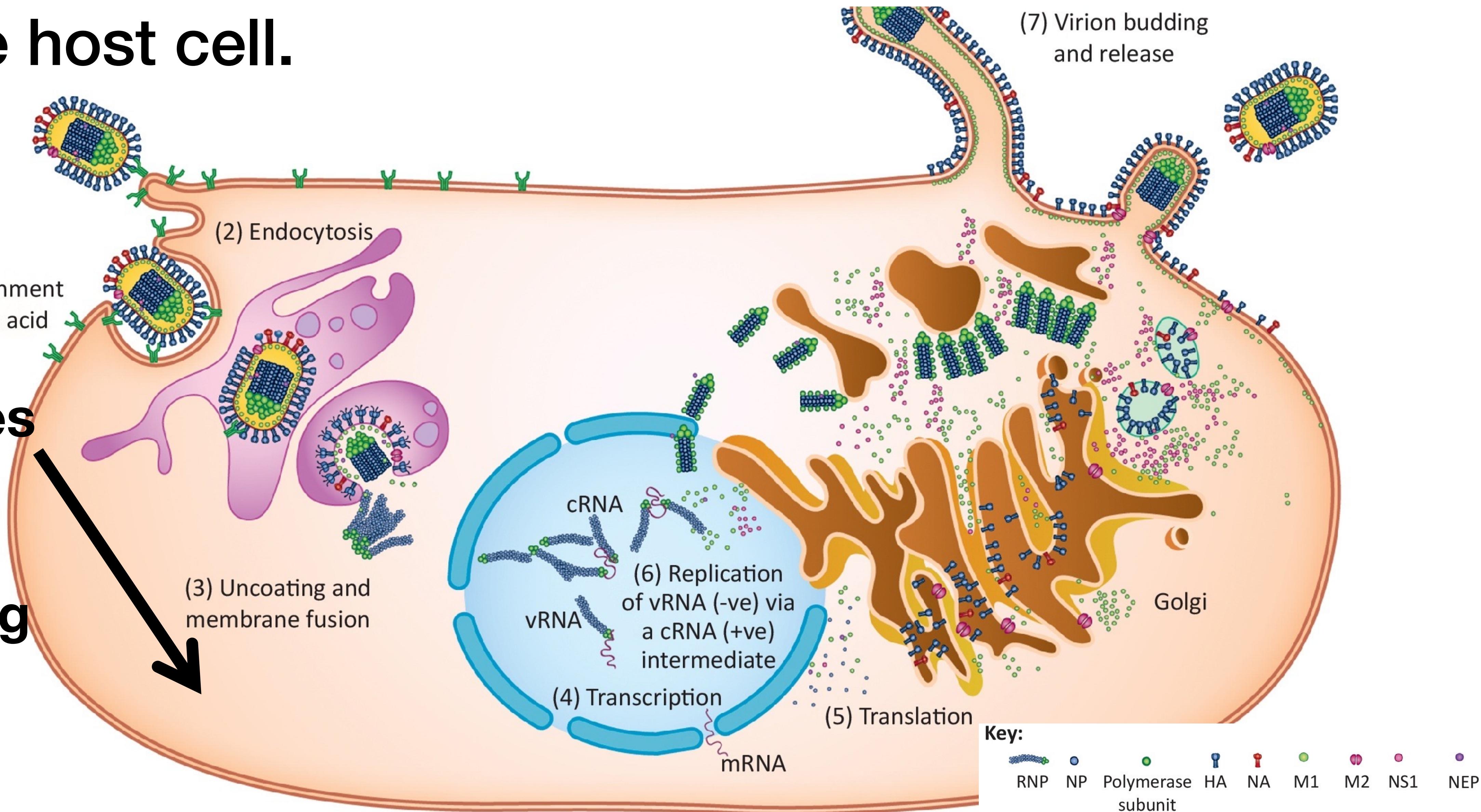
* human lower
lungs have
some α -2,3 SAs

Hemagglutinin binds the sialic acid receptor, triggering receptor mediated endocytosis

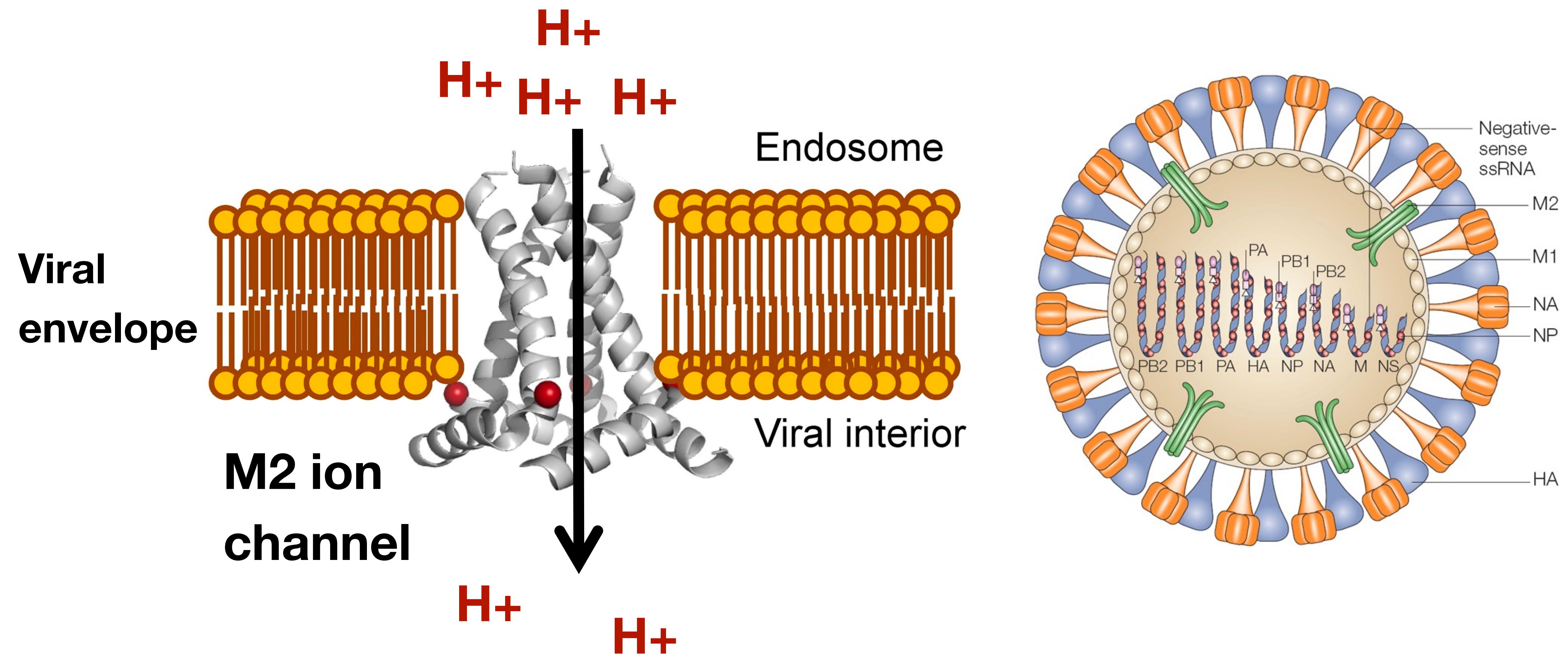


The endosome will acidify during trafficking to the nucleus. Influenza has co-opted this process for release into the host cell.

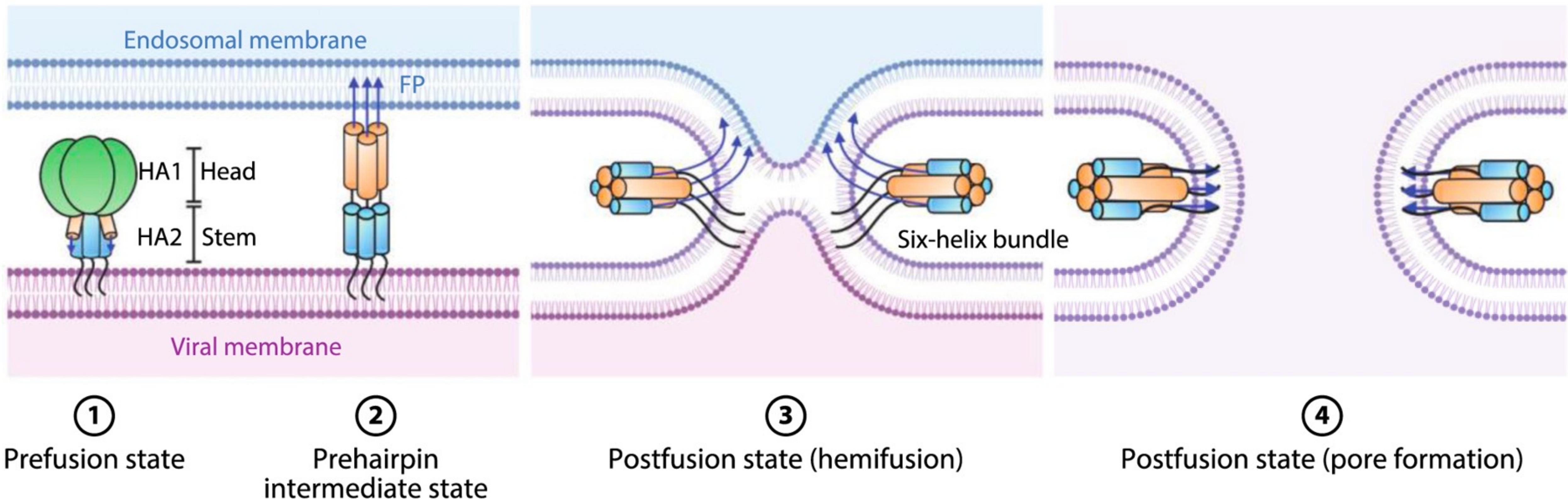
pH
decreases
during
nuclear
trafficking



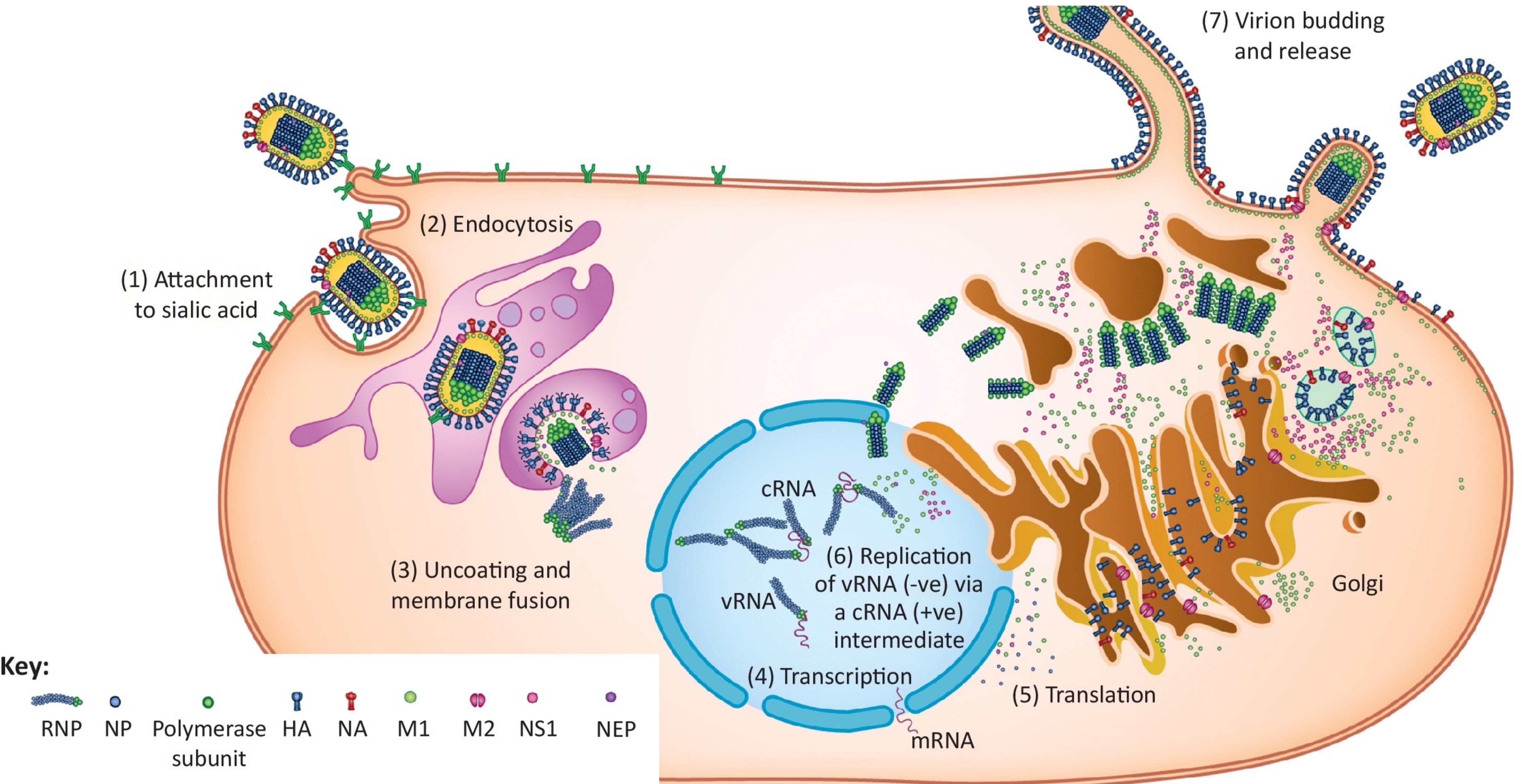
1. The M2 ion channel pumps protons into the virion core, allowing the vRNPs to dissociate from the capsid



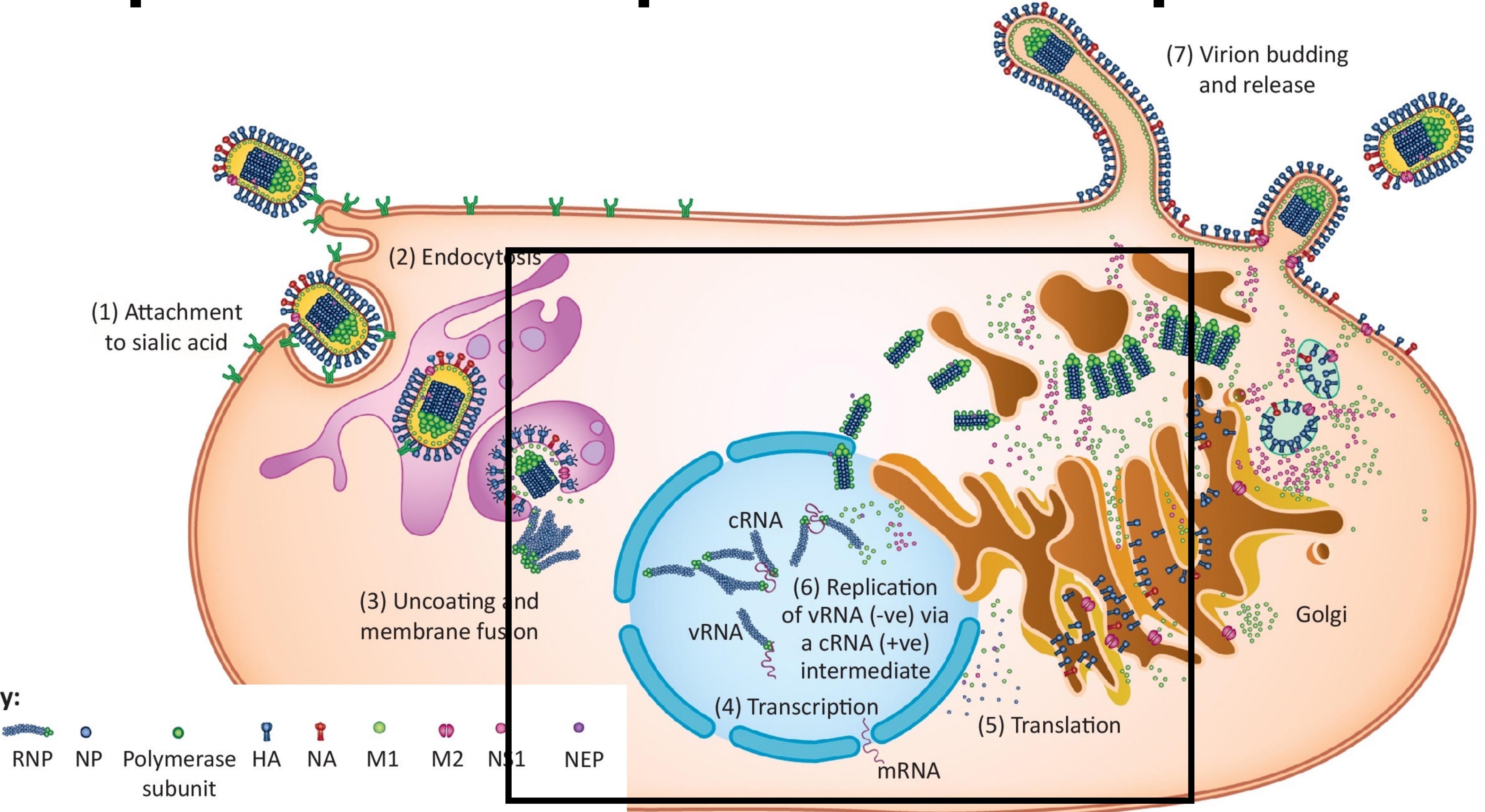
2. Acidification triggers irreversible conformational changes in HA that trigger fusion of the viral and endosomal membrane, allowing the virion contents to be released into the cytoplasm



vRNPs are released into the cytoplasm, and trafficked to the nucleus via a nuclear localization signal in NP

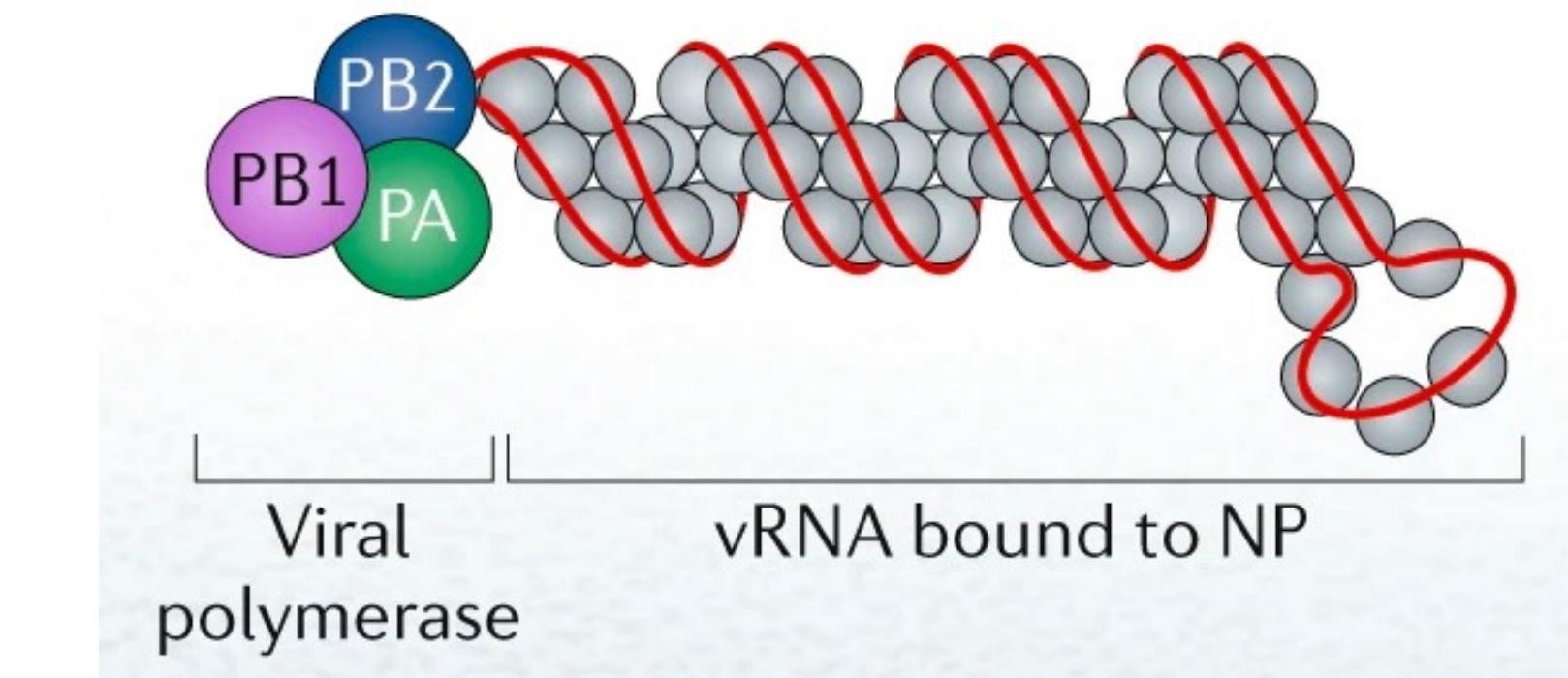


Step 2: transcription and replication

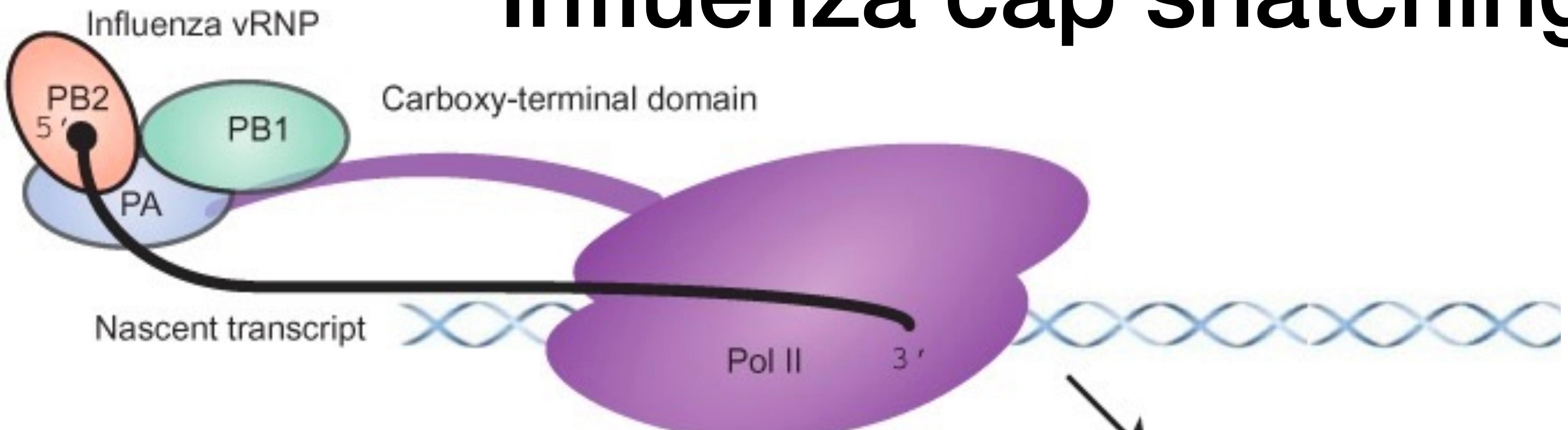


Unique features of influenza transcription and genome replication

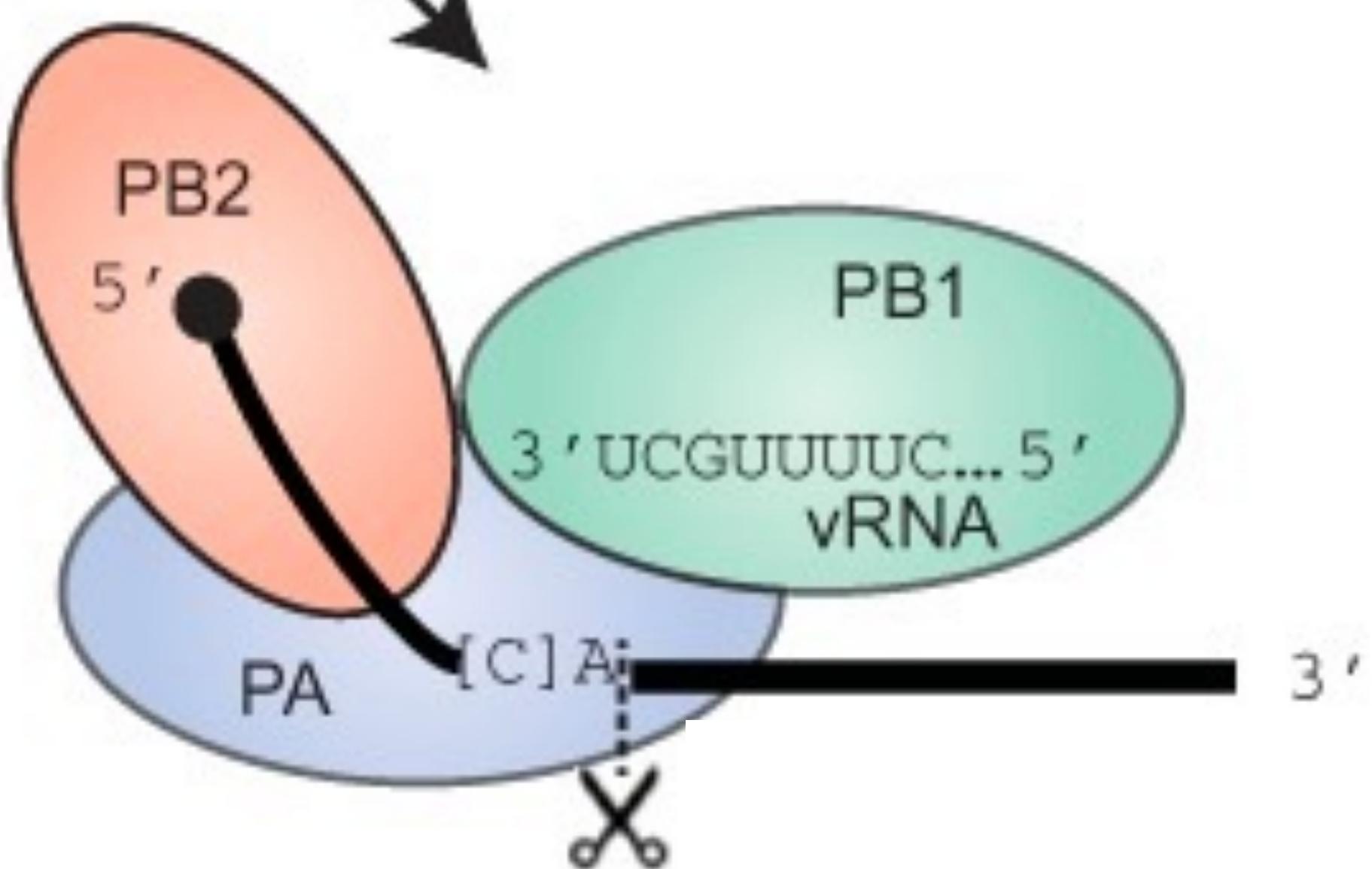
1. The influenza genome is negative sense, so no existing host machinery can transcribe influenza genomic RNA into mRNA. Influenza must provide its own polymerase for transcription.
2. Influenza polymerases can't generate m₇ caps, which are necessary for translation. Influenza has evolved a unique mechanism called “cap snatching”



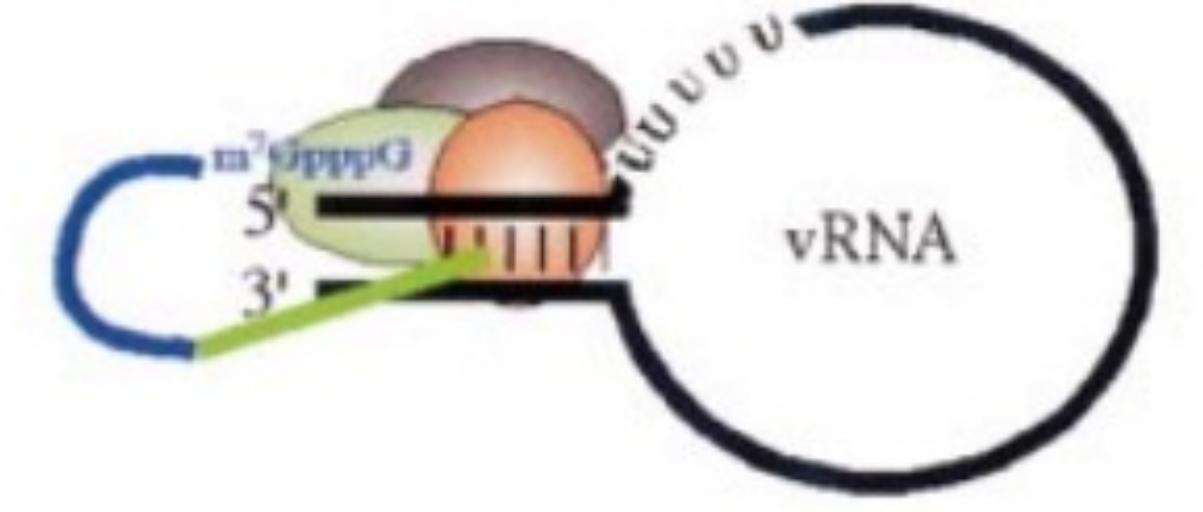
Influenza cap snatching



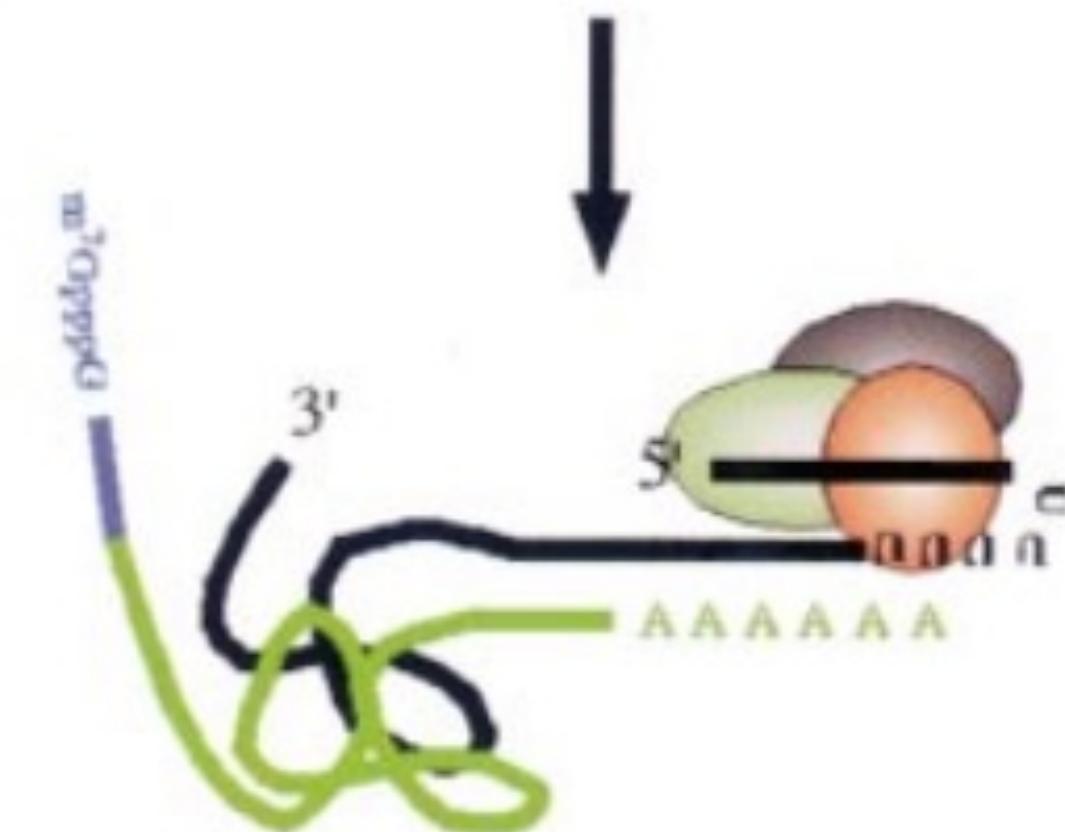
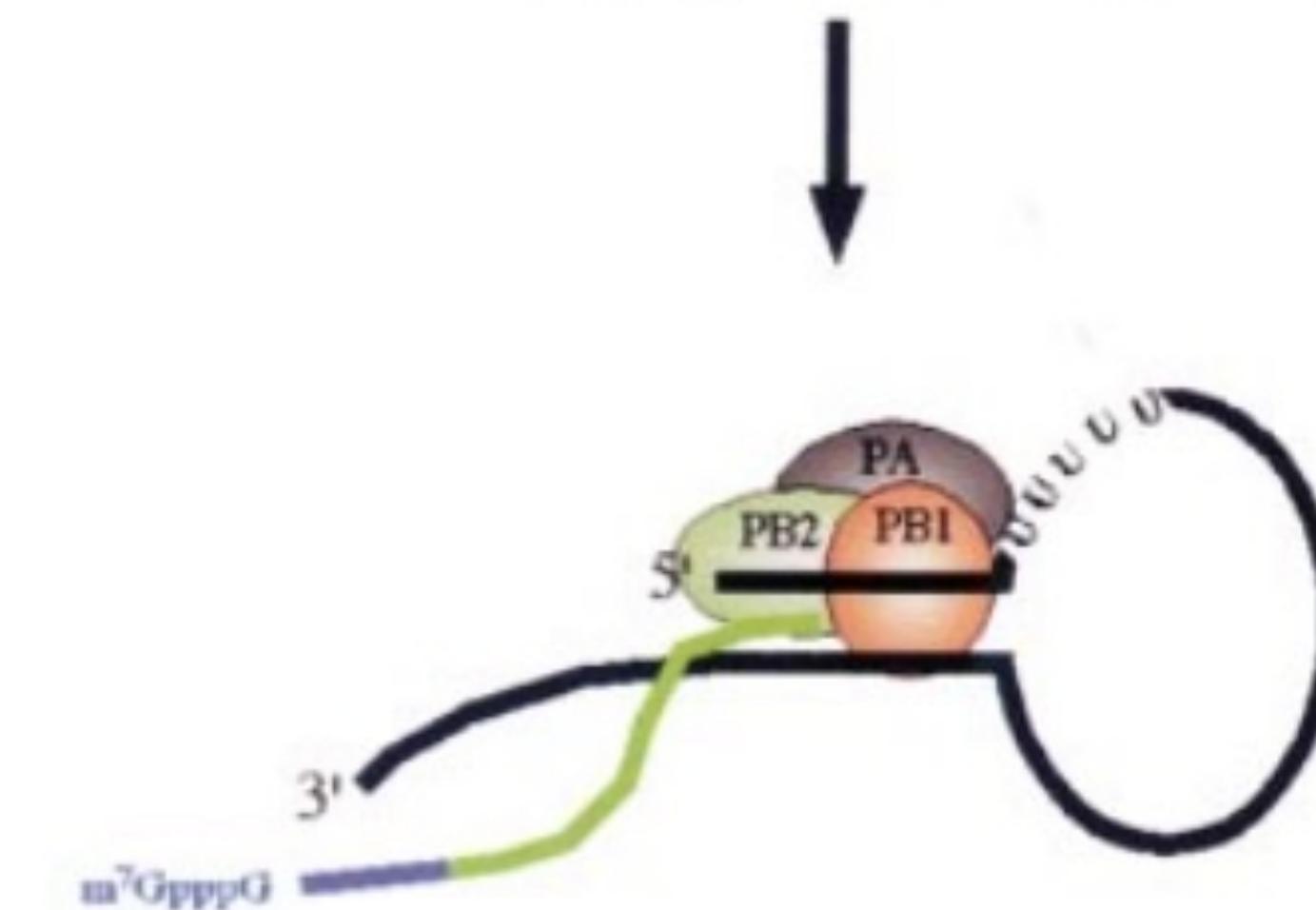
1. Influenza vRNP interacts with host RNA polymerase II, and PB2 binds the m₇ cap
2. PA is an endonuclease that cleaves the m₇ cap, generating a capped, mRNA fragment from a host transcript
3. PB1 uses this as a primer to transcribe the viral RNA into mRNA.
4. PolyA tails are added by polymerase stuttering at a conserved, polyU tract at the end of the transcript



PolyA tails are added
when the polymerase
stutters at a polyU tract at
the end of the transcript

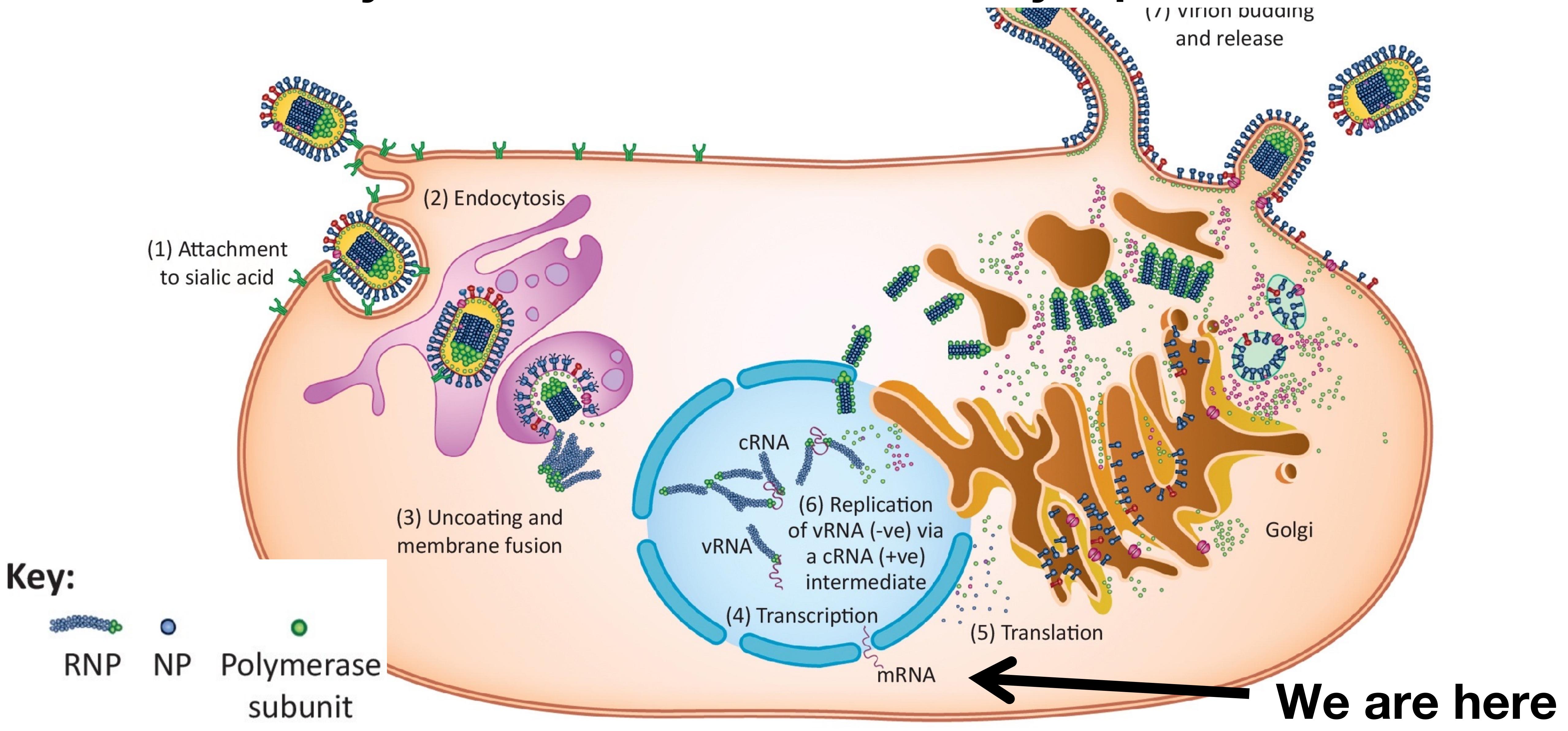


Cap-primed initiation



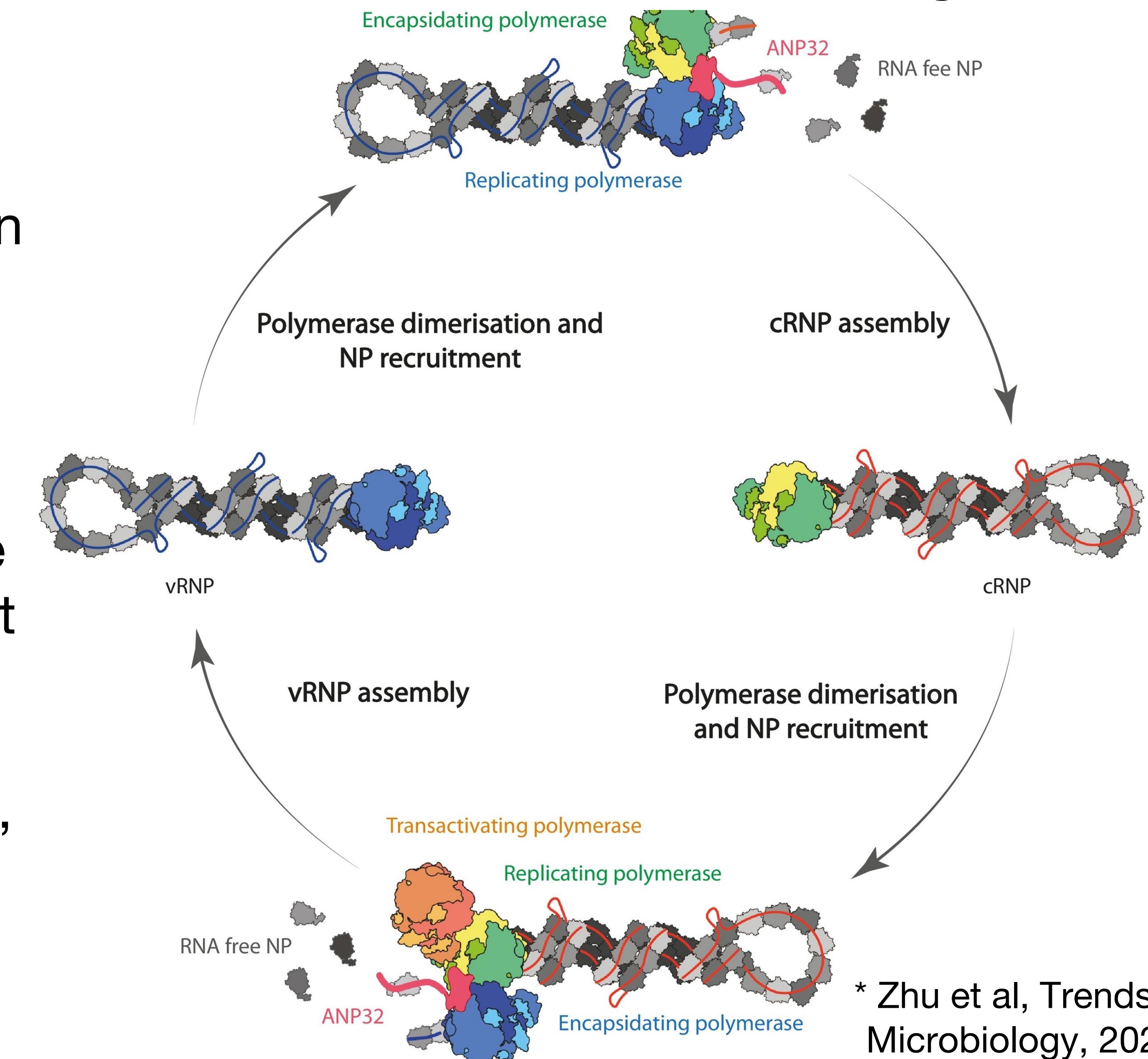
Polyadenylation

Complete mRNA transcripts are exported and translated by host ribosomes in the cytoplasm



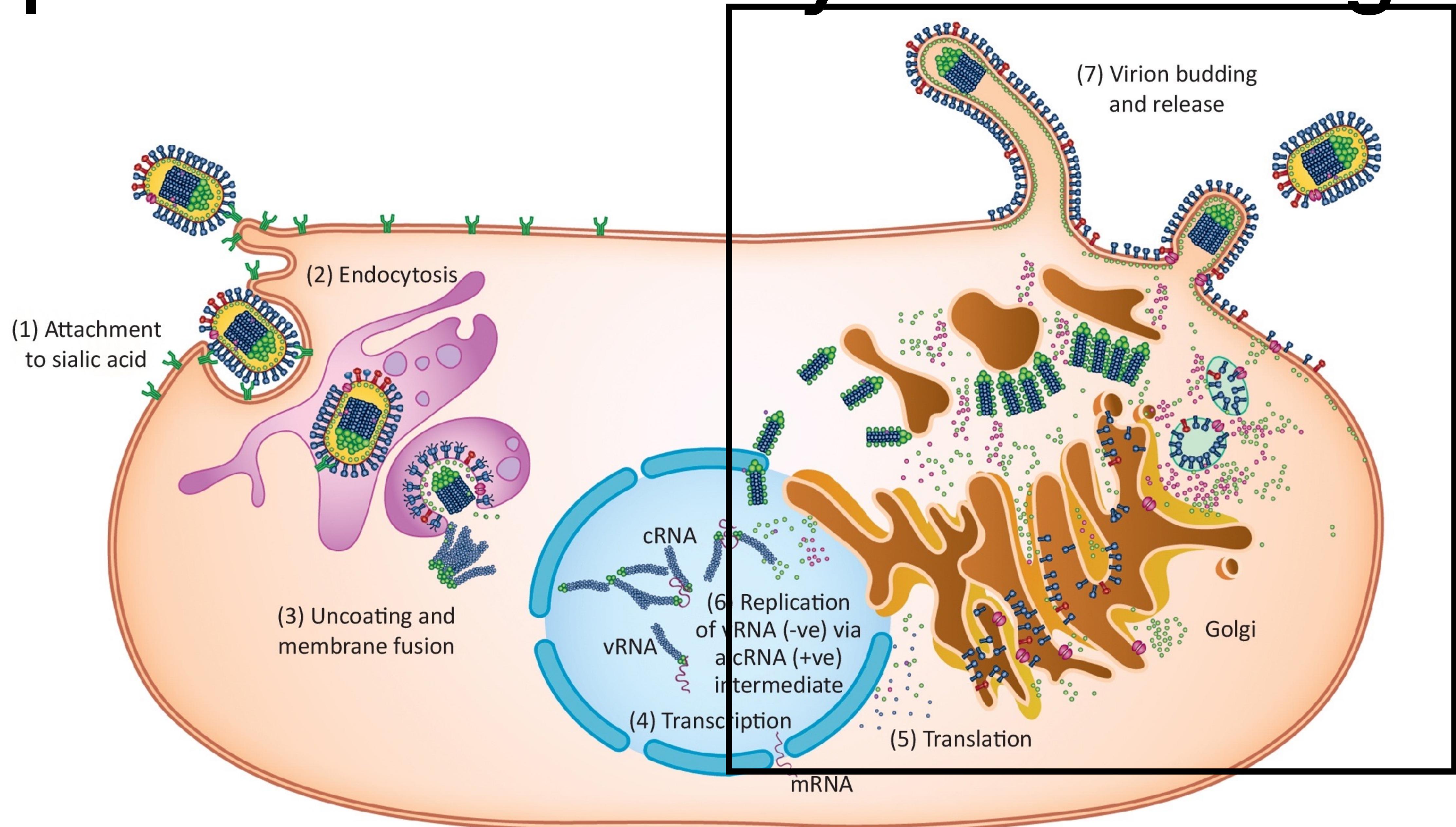
Viral genome replication is complex, and poorly understood

- Influenza viral genome replication occurs when sufficient polymerase and nucleoproteins accumulate in nucleus
- Replication requires NP, multiple polymerase complexes, and host factors
- Replication occurs via a + sense, complementary RNA intermediate (cRNA)

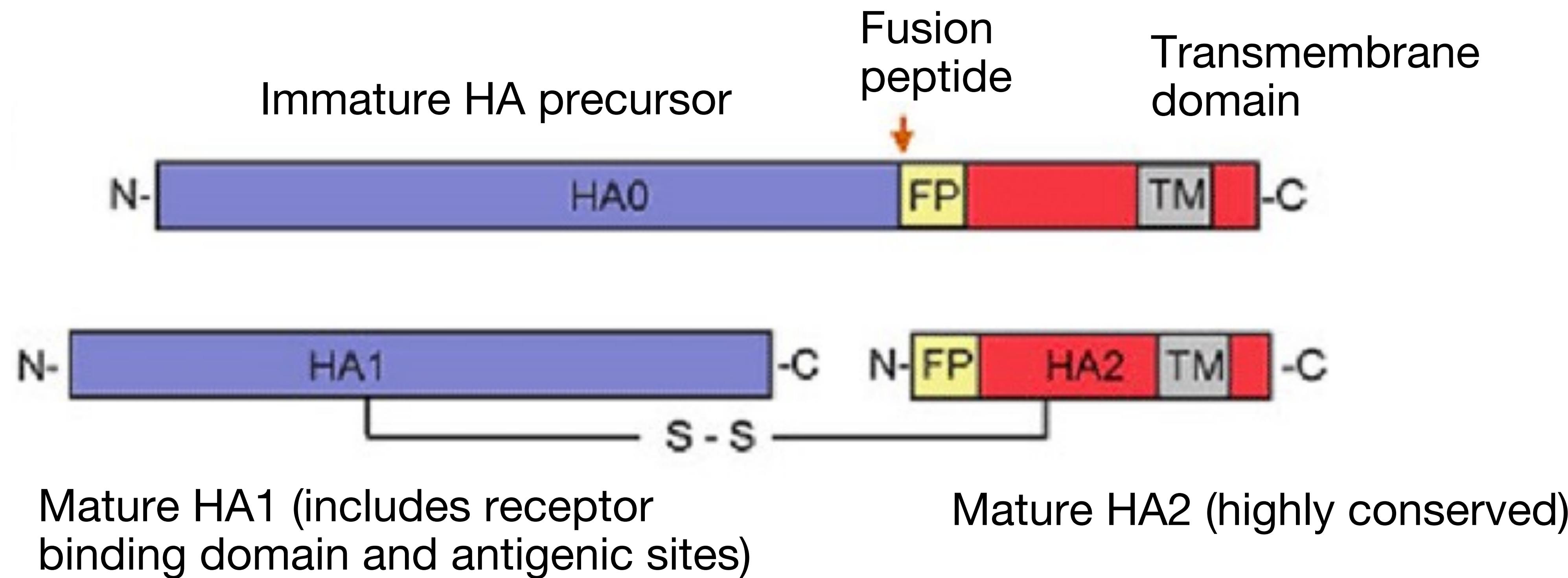


* Zhu et al, Trends in Microbiology, 2022

Step 3: virion assembly and budding



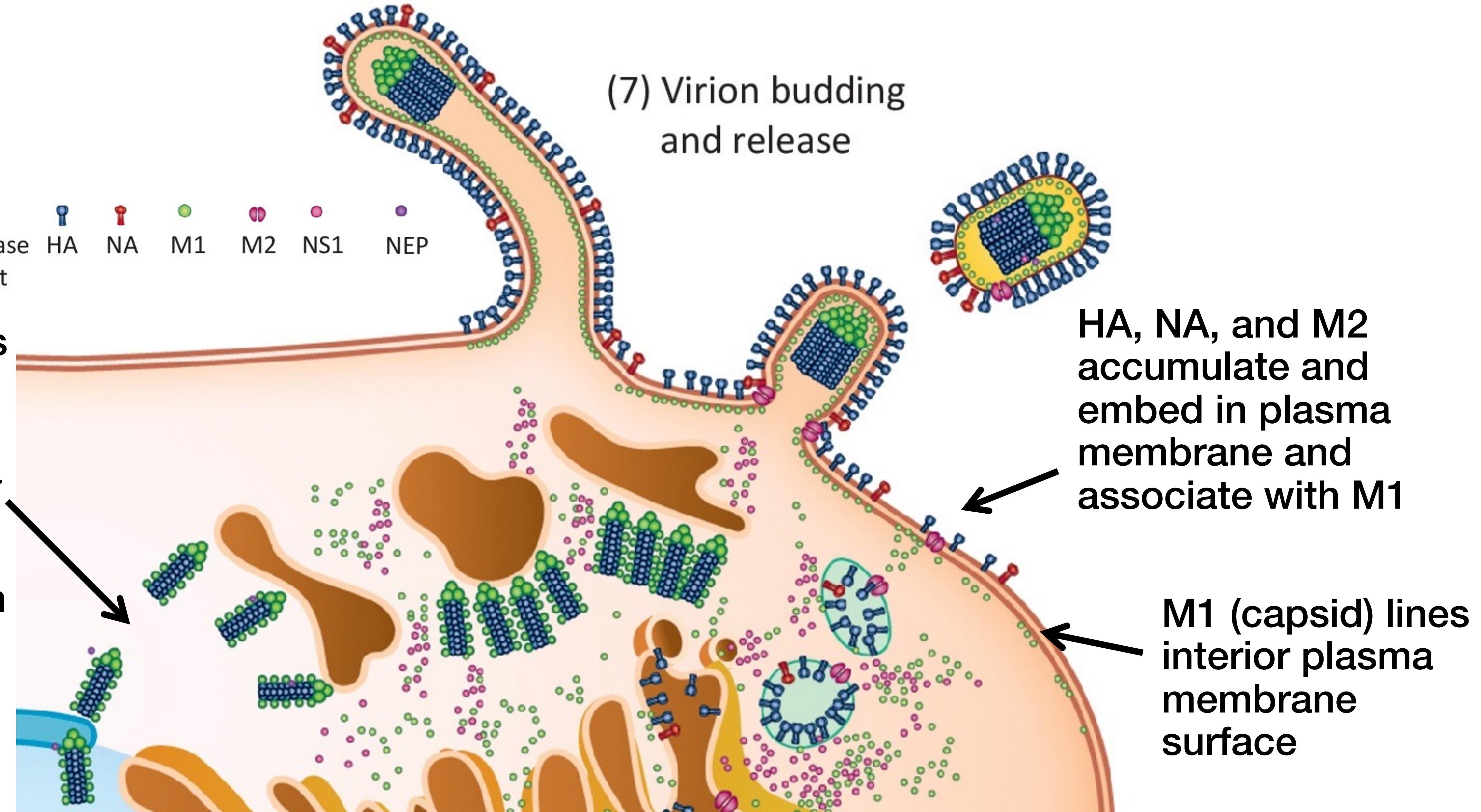
Hemagglutinin is cleaved during processing in the golgi by trypsin-like proteases. Cleavage is necessary for fully functional HA. Uncleaved HA cannot unfold at low pH



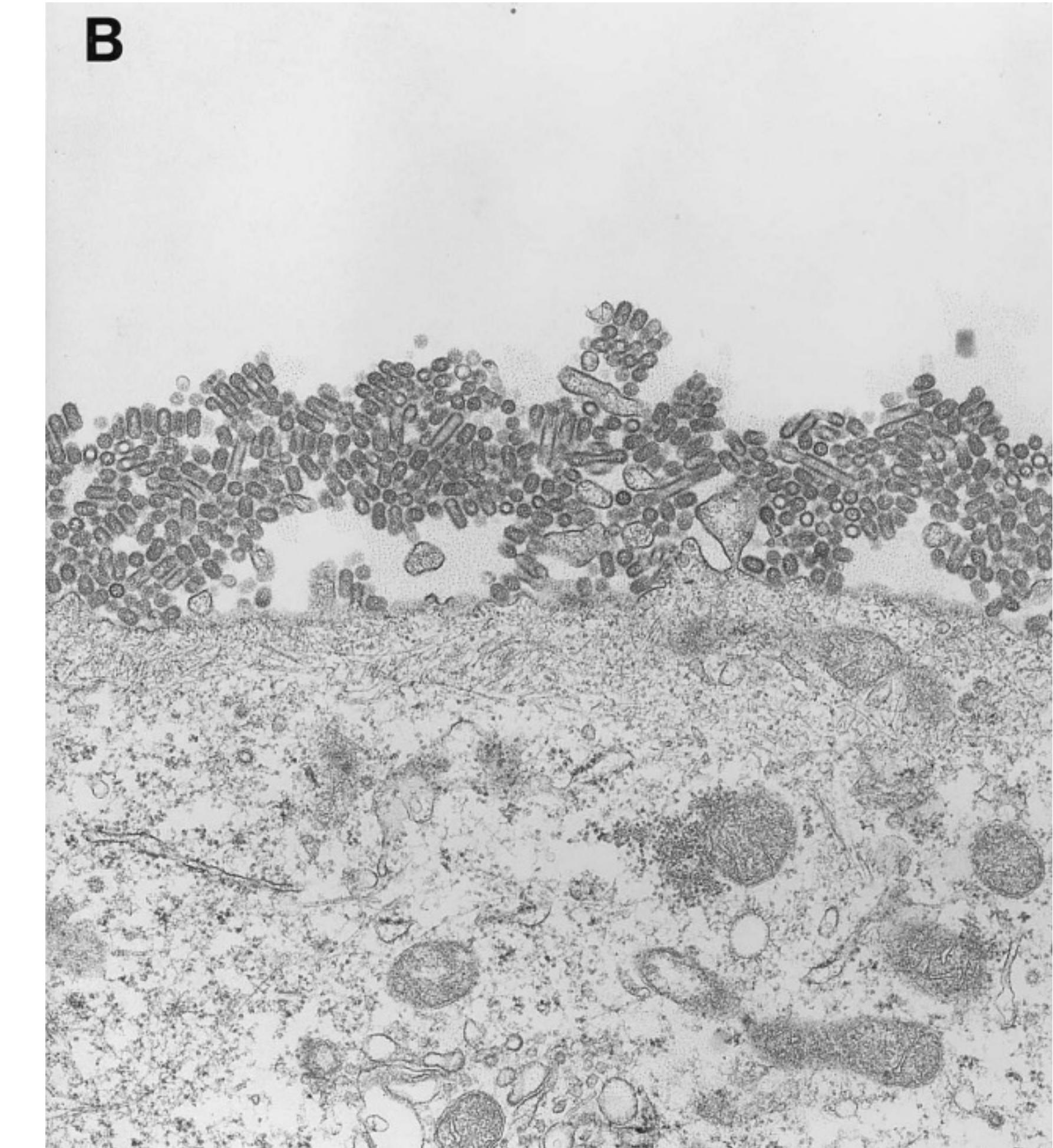
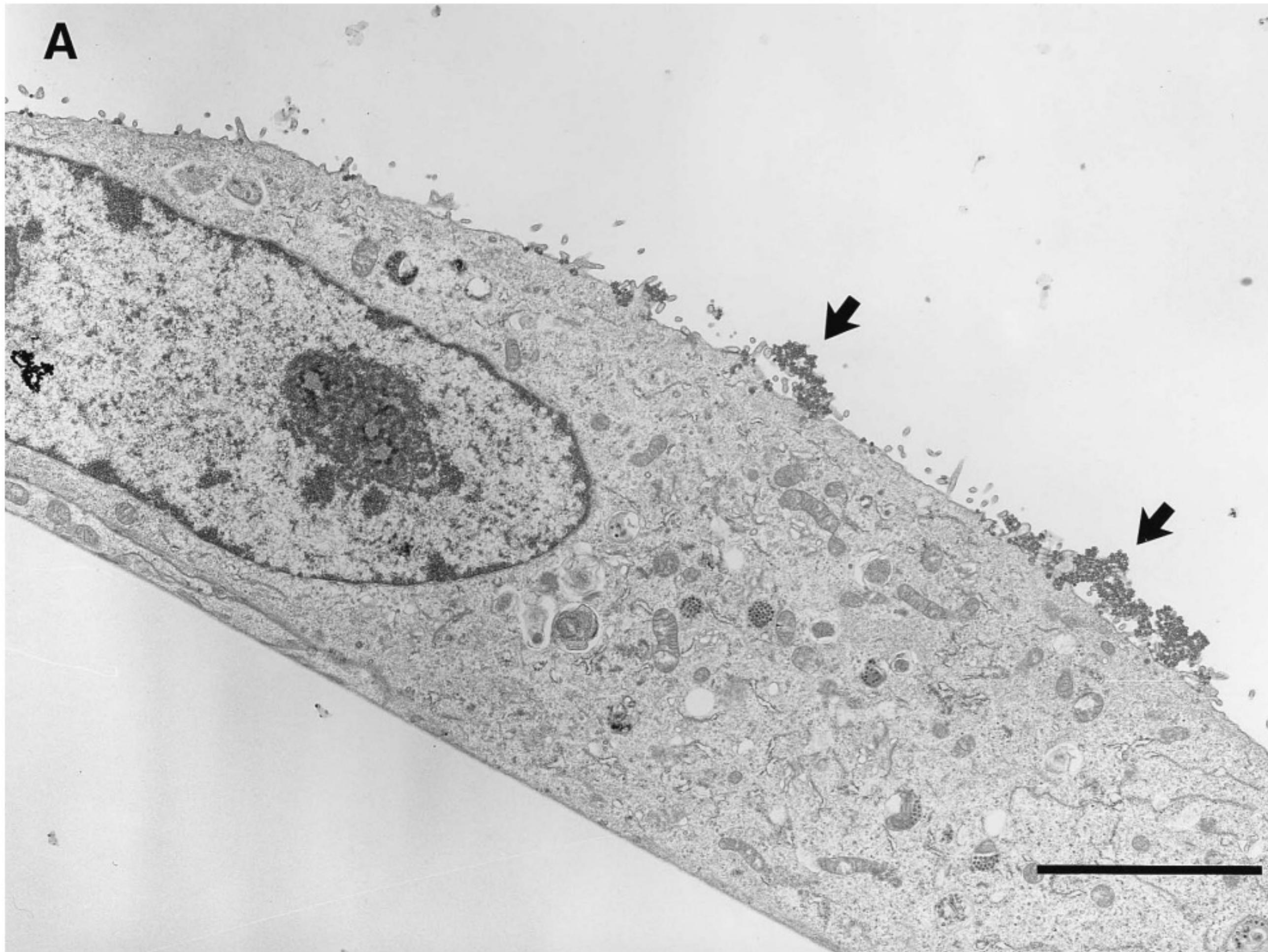
Virions assemble at lipid rafts on plasma membrane, and bud from cell surface via NA cleavage

Key:

RNP	NP	Polymerase subunit	HA	NA	M1	M2	NS1	NEP
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Without NA, influenza virions aggregate on the cell surface

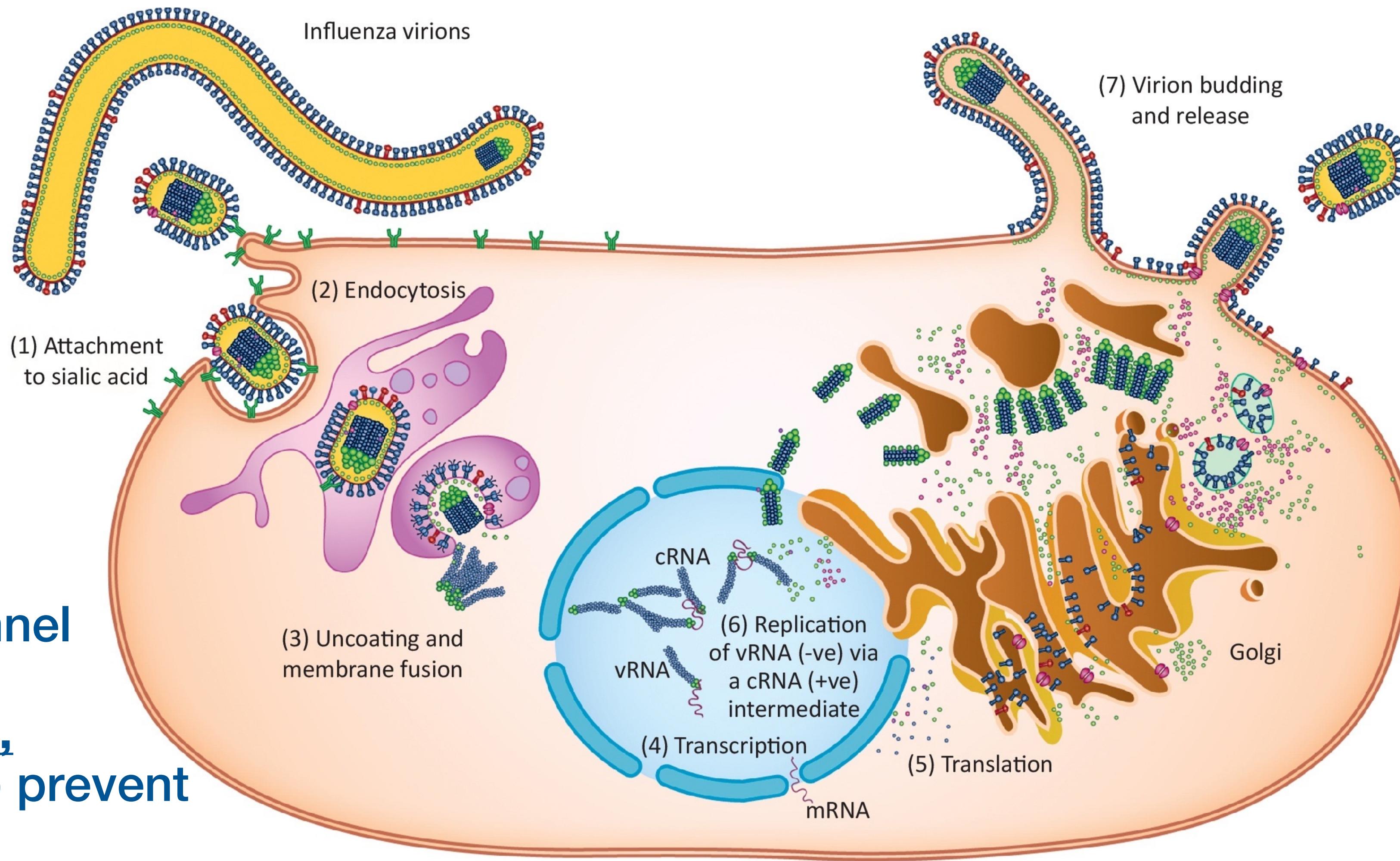


* Liu et al, Journal of Virology, 1995

Multiple parts of the life cycle are targeted by antivirals and immune responses

Antibodies
bind HA,
preventing
receptor
binding or
fusion

M2 ion channel
inhibitors
**(amantidine,
rimantidine)** prevent
uncoating



Neuraminidase
inhibitors
(oseltamivir)
prevent sialic
acid cleavage

Influenza NS1
antagonizes
interferon and
RIG-I

NP antagonizes
MxA

Recap of the influenza life cycle

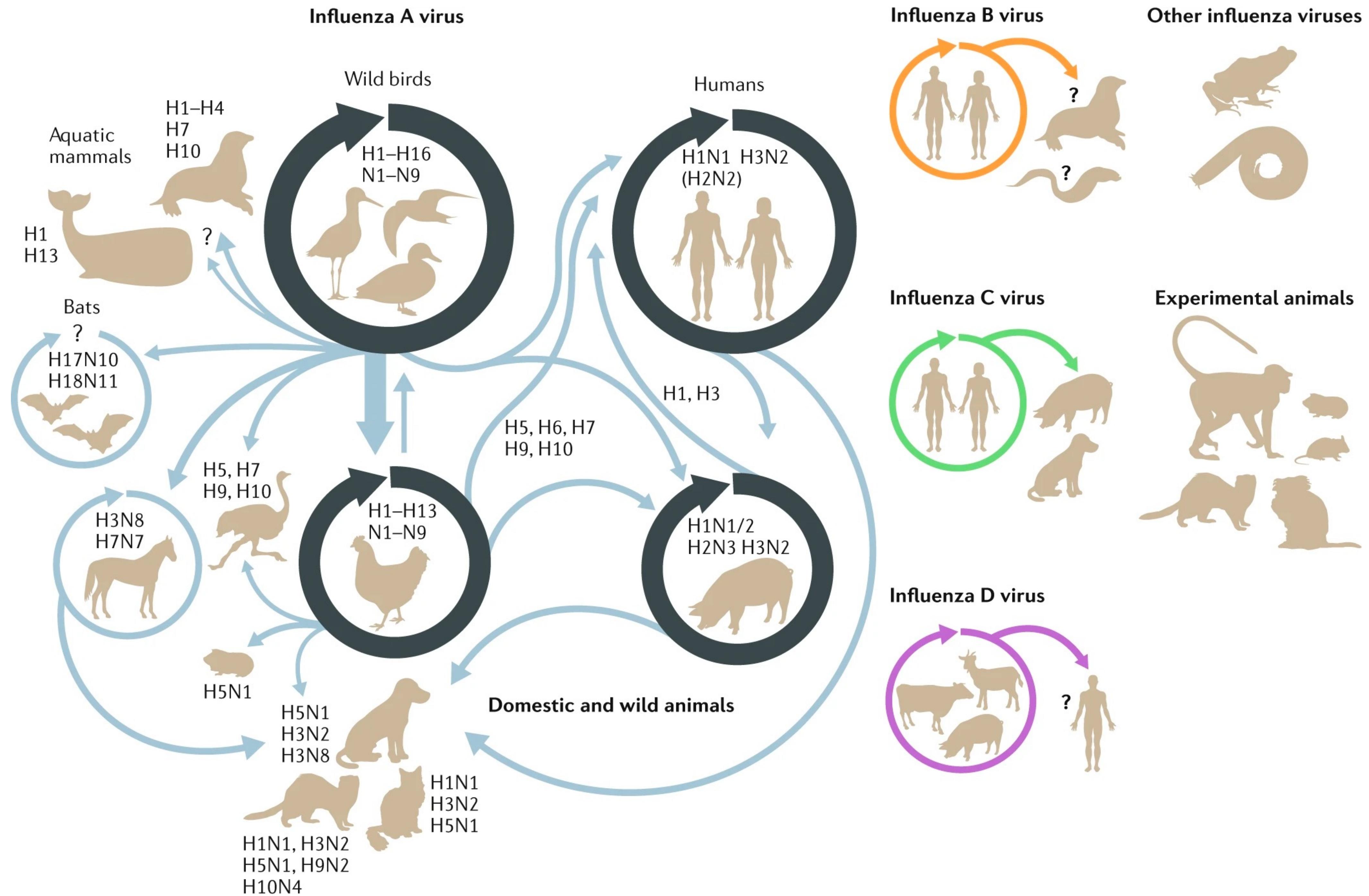
1. Influenza binds a sialic acid receptor, which varies across different host species
2. Receptor binding triggers receptor mediated endocytosis and endosomal acidification. Acidification causes an irreversible conformational change in HA, triggering membrane fusion. M2 pumps ions into the virion dissociating M1 from vRNPs, allowing them to be released into the nucleus
3. vRNPs are trafficked to the nucleus. Transcription proceeds via cap snatching. PB2 binds host m7 caps, PA cleaves the cap, and PB1 uses that piece of RNA as a primer for mRNA transcription.
4. mRNA is exported and translated by host ribosomes in the ER (HA, NA, and M2), or in the cytoplasm (others).
5. Polymerase proteins are re-imported into the nucleus, where they accumulate to trigger a switch from transcription to replication. The polymerase generates a positive sense, complementary RNA (cRNA), which is used as a template for more negative sense, genomic RNA.
6. Mature membrane proteins (HA, NA, and M2) traffic to lipid rafts and embed in plasma membrane. M1 lines the interior of the plasma membrane, and mature vRNPs associate with M1.
7. Virions bud by pinching off from the host cell, and are prevented from re-binding by NA cleavage.
8. Antivirals target cell entry (M2 ion channel inhibitors) and exit (neuraminidase inhibitors)

Outline:

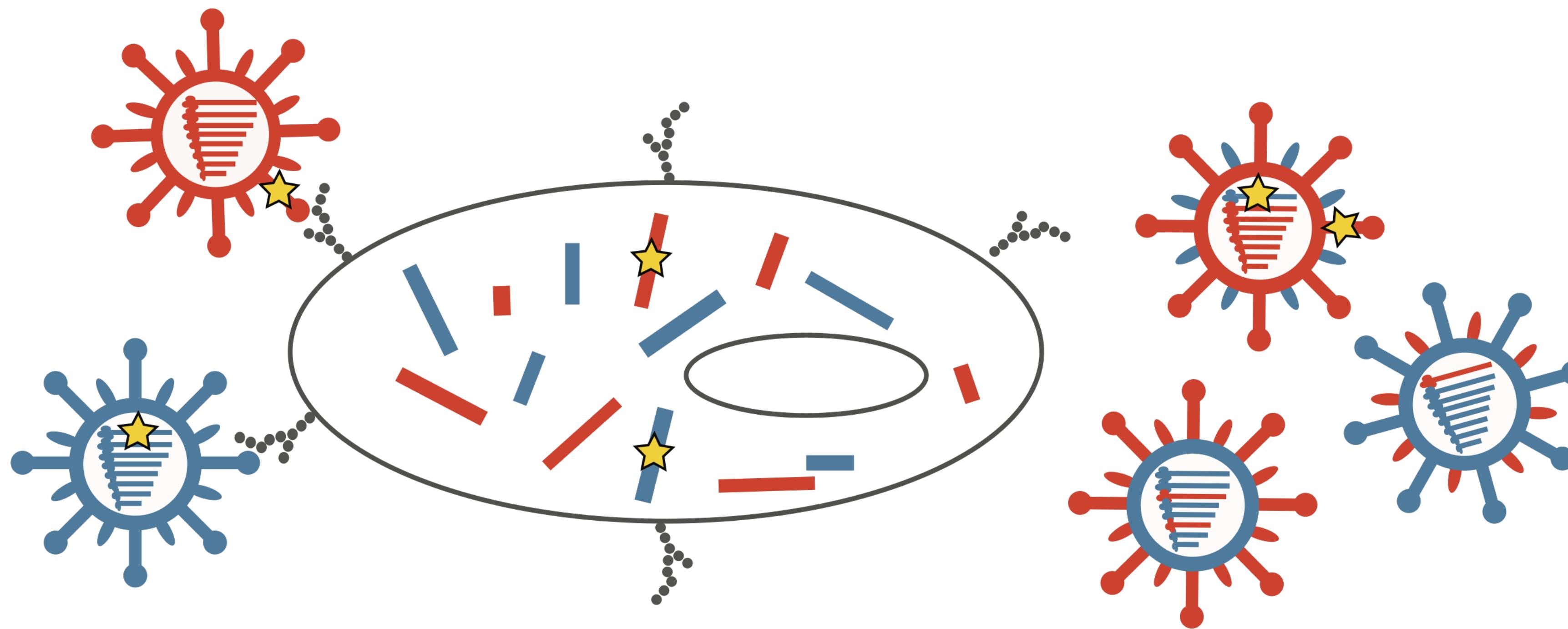
1. Influenza structure and life cycle
2. **Influenza host ecology, evolution, pandemic virus formation, and antigenic drift**
3. Emerging public health issues in influenza
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Influenza A viruses naturally circulate in wild,
aquatic, migratory birds (ducks, geese, swans)

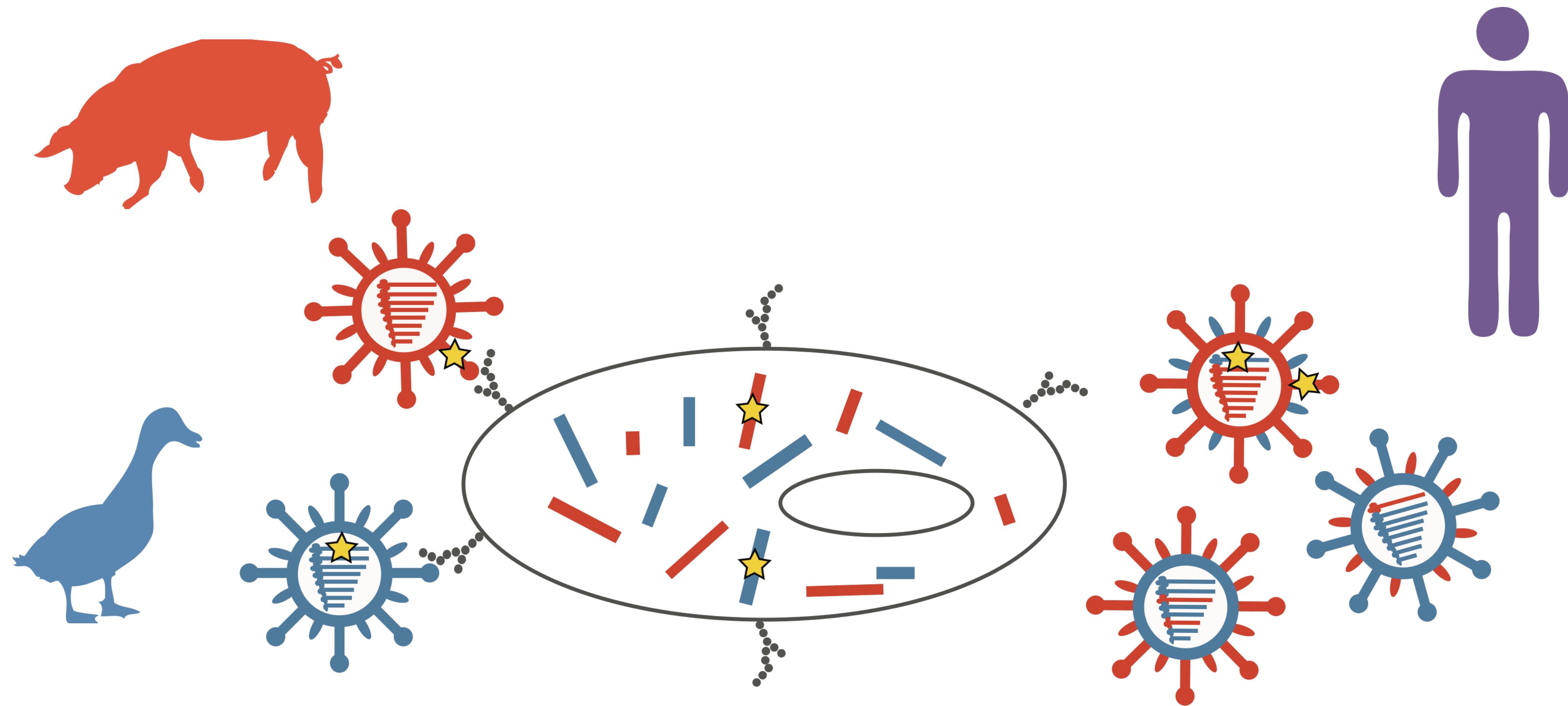


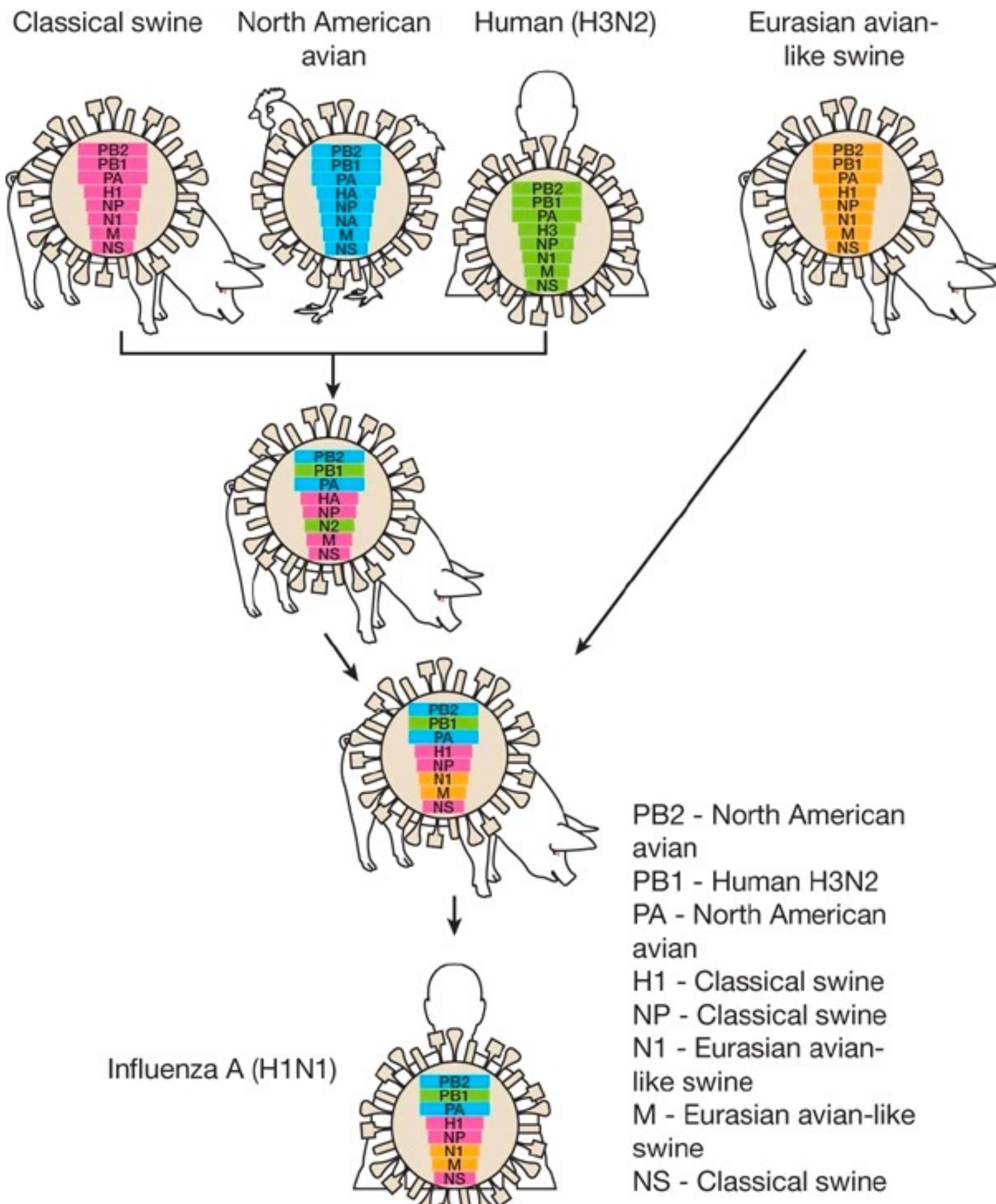


Influenza viruses mix via “reassortment” which can generate novel viruses



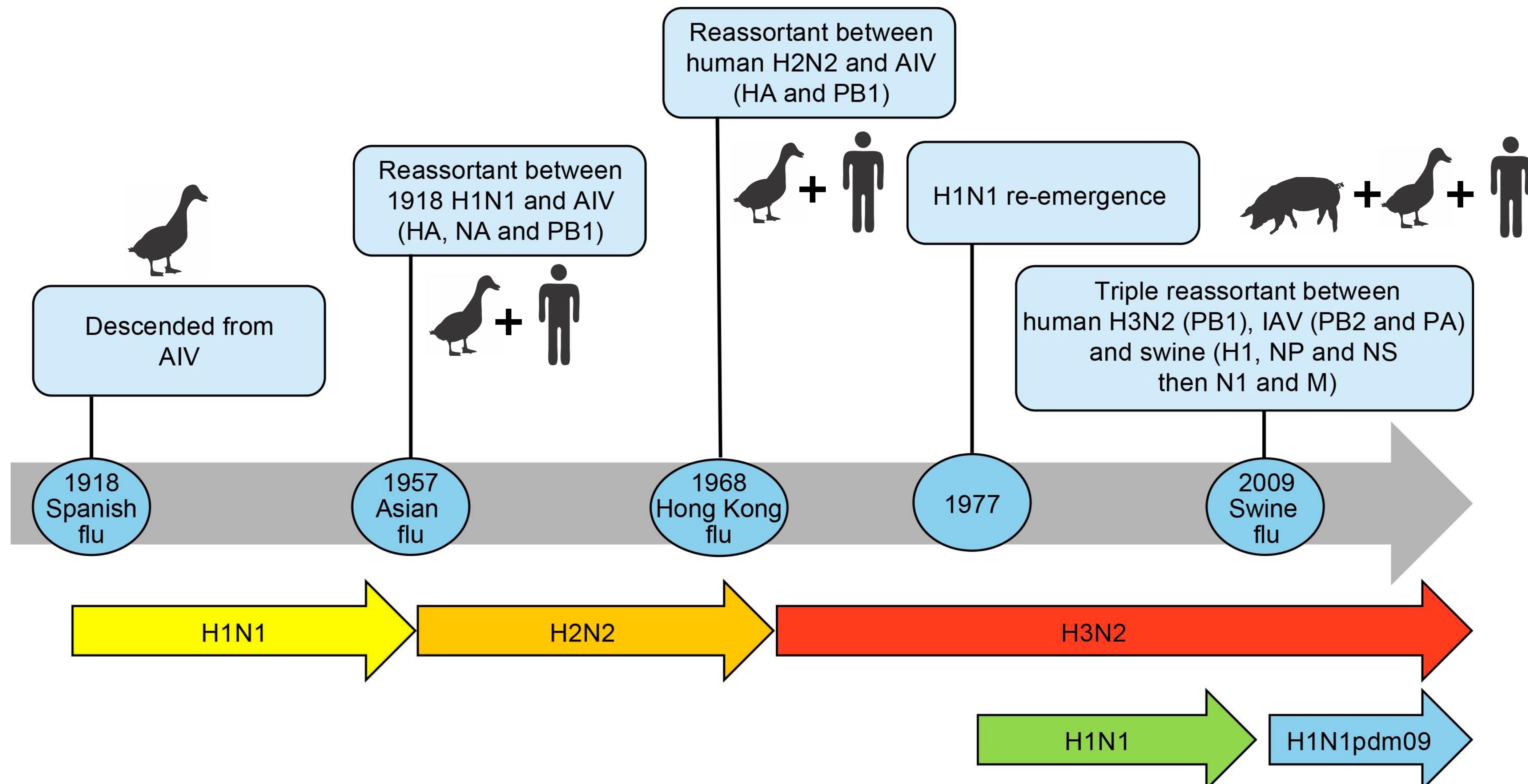
When reassortment occurs between viruses from different hosts, it can cause “antigenic shift”





The 2009 “swine flu” pandemic was the result of reassortment between bird, human, and pig viruses!

Influenza pandemics and panzootics arise from reassortment and introduction into new animal populations

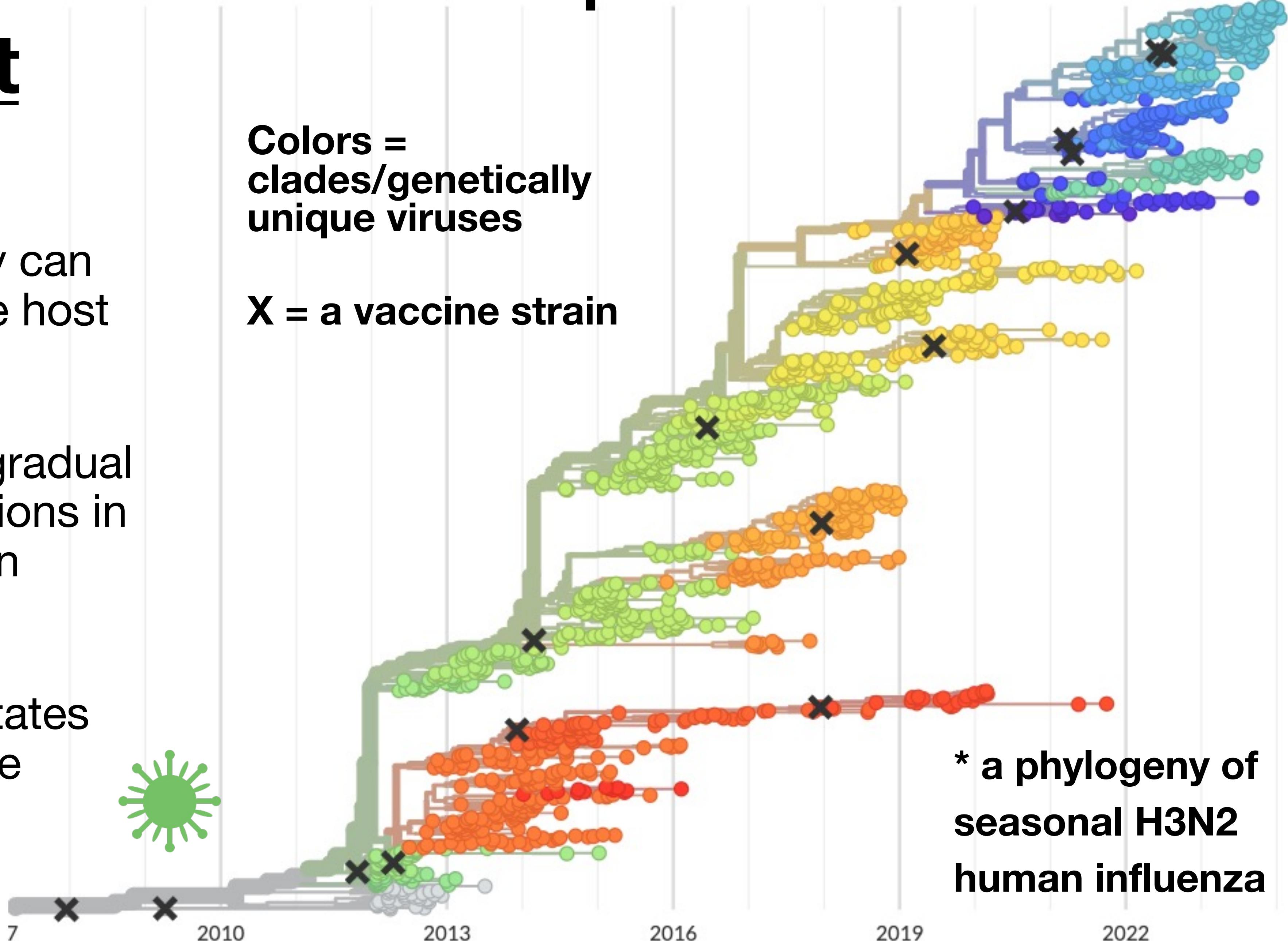
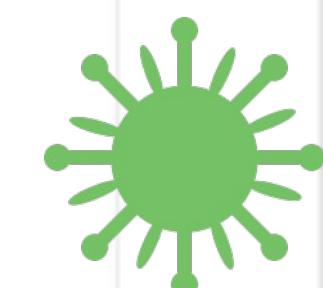


Influenza vaccination and the problem of antigenic drift

- RNA viruses have high mutation rates, so they can rapidly evolve to evade host immunity
- **Antigenic drift** is the gradual accumulation of mutations in HA to evade population immunity
- Antigenic drift necessitates yearly influenza vaccine updates

Colors =
clades/genetically
unique viruses

X = a vaccine strain



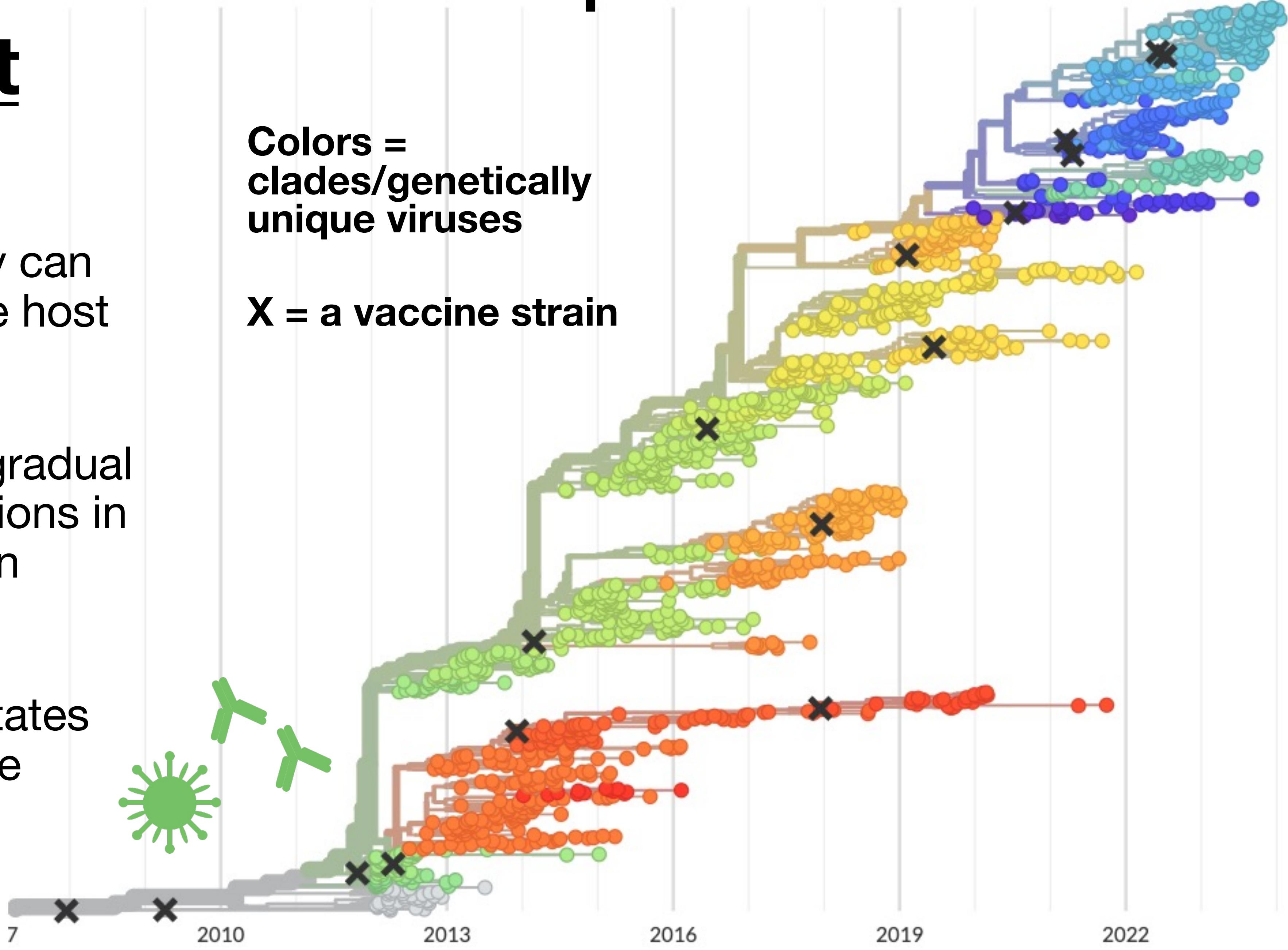
* a phylogeny of
seasonal H3N2
human influenza

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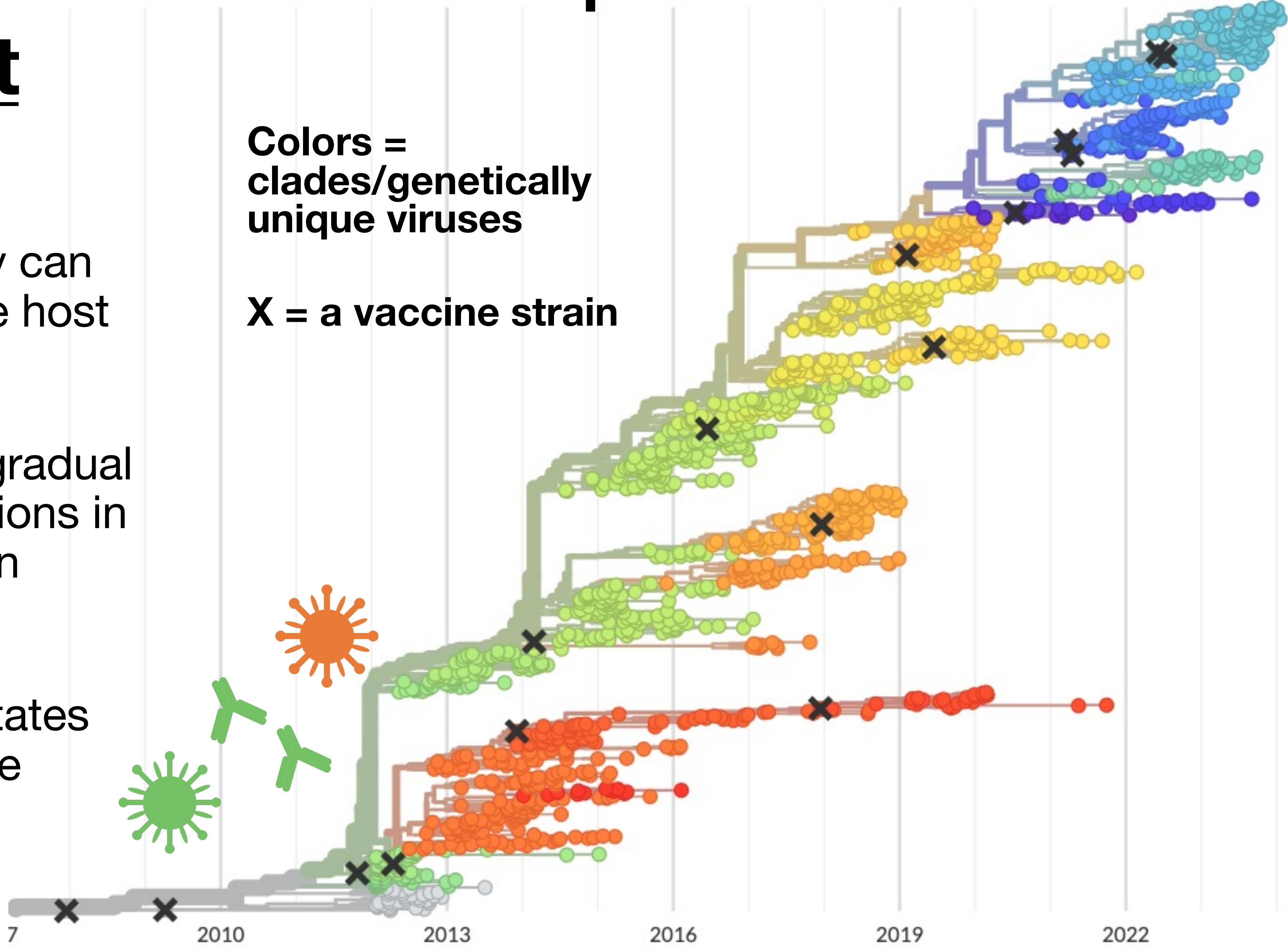


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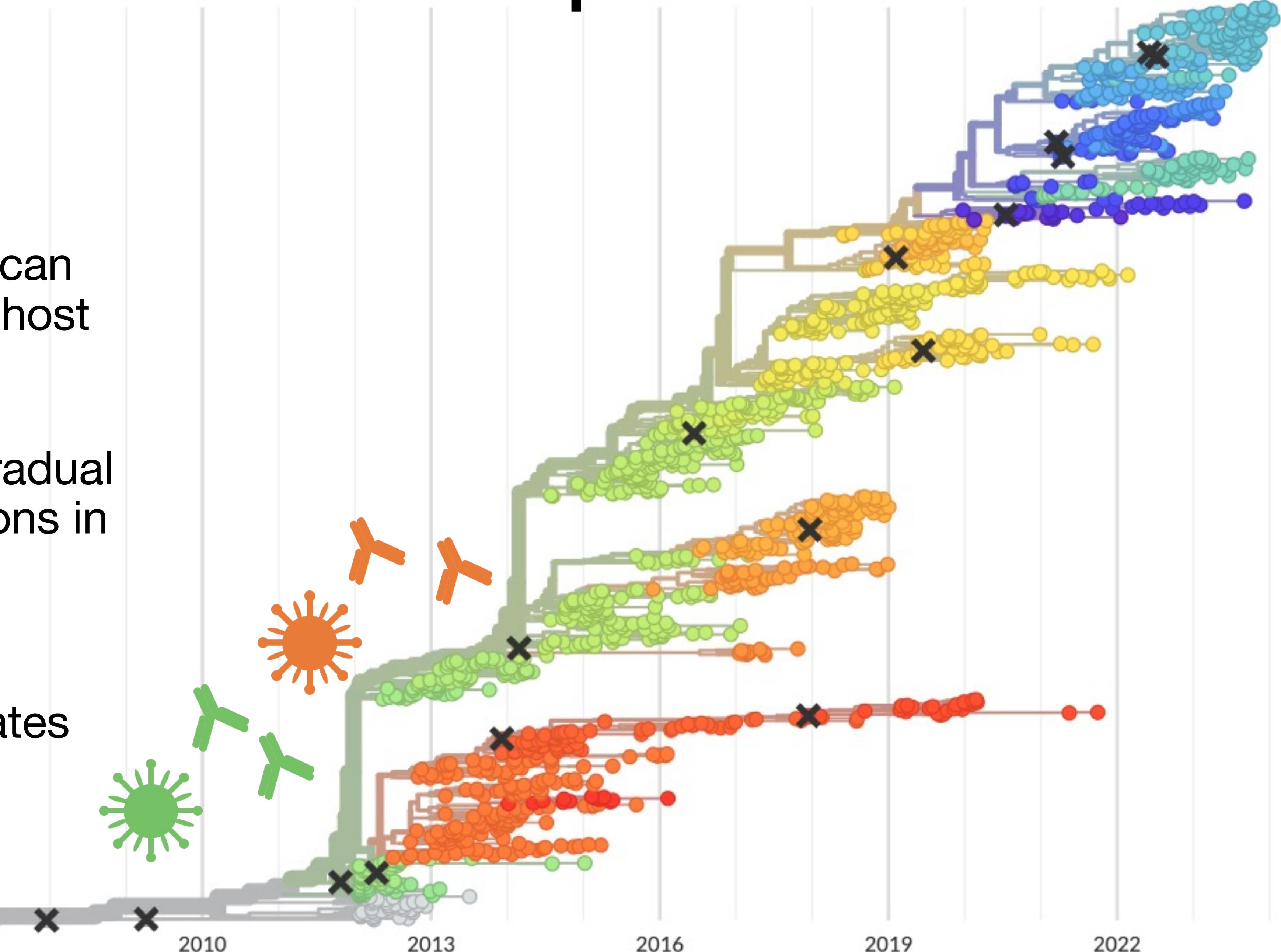
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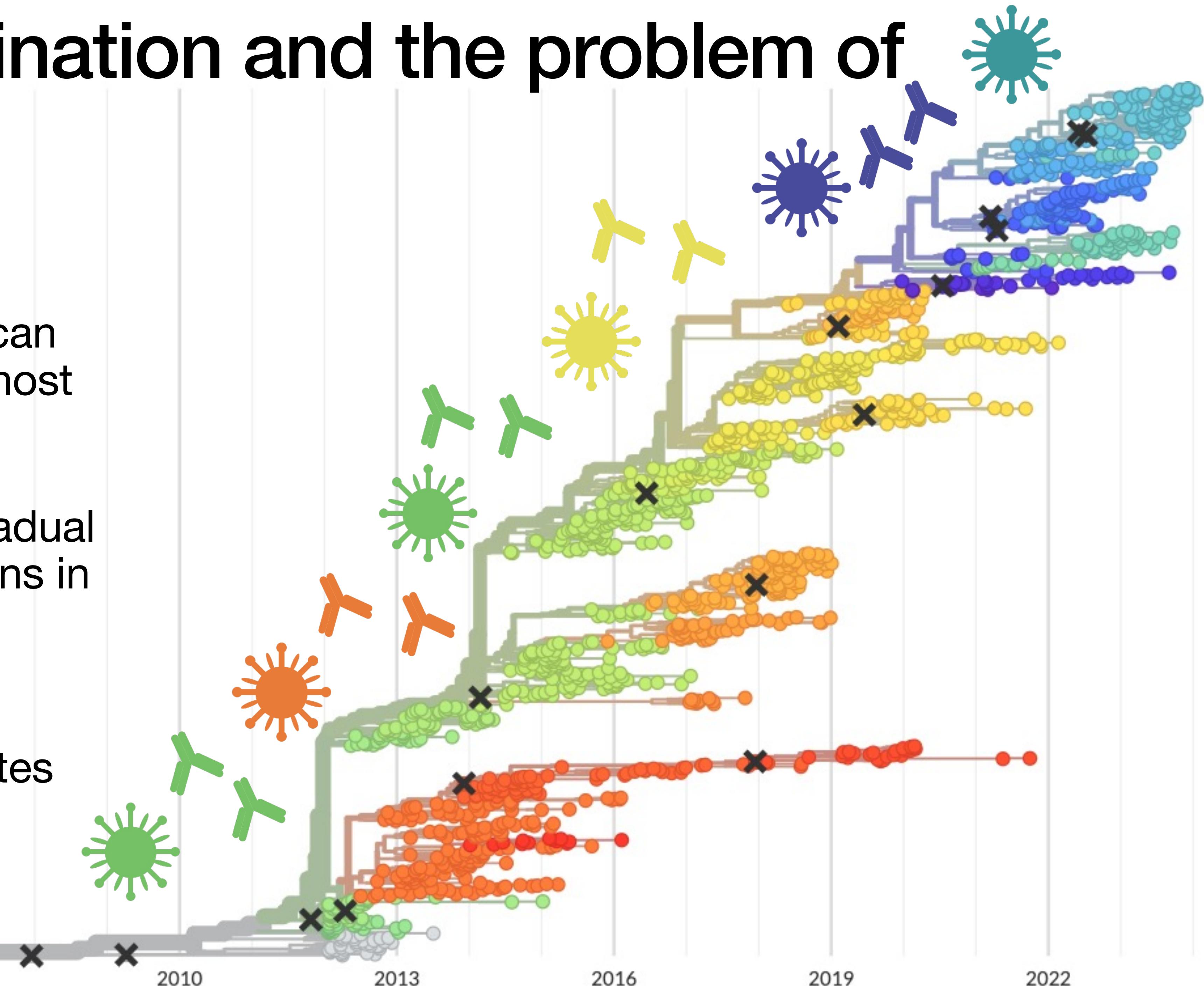
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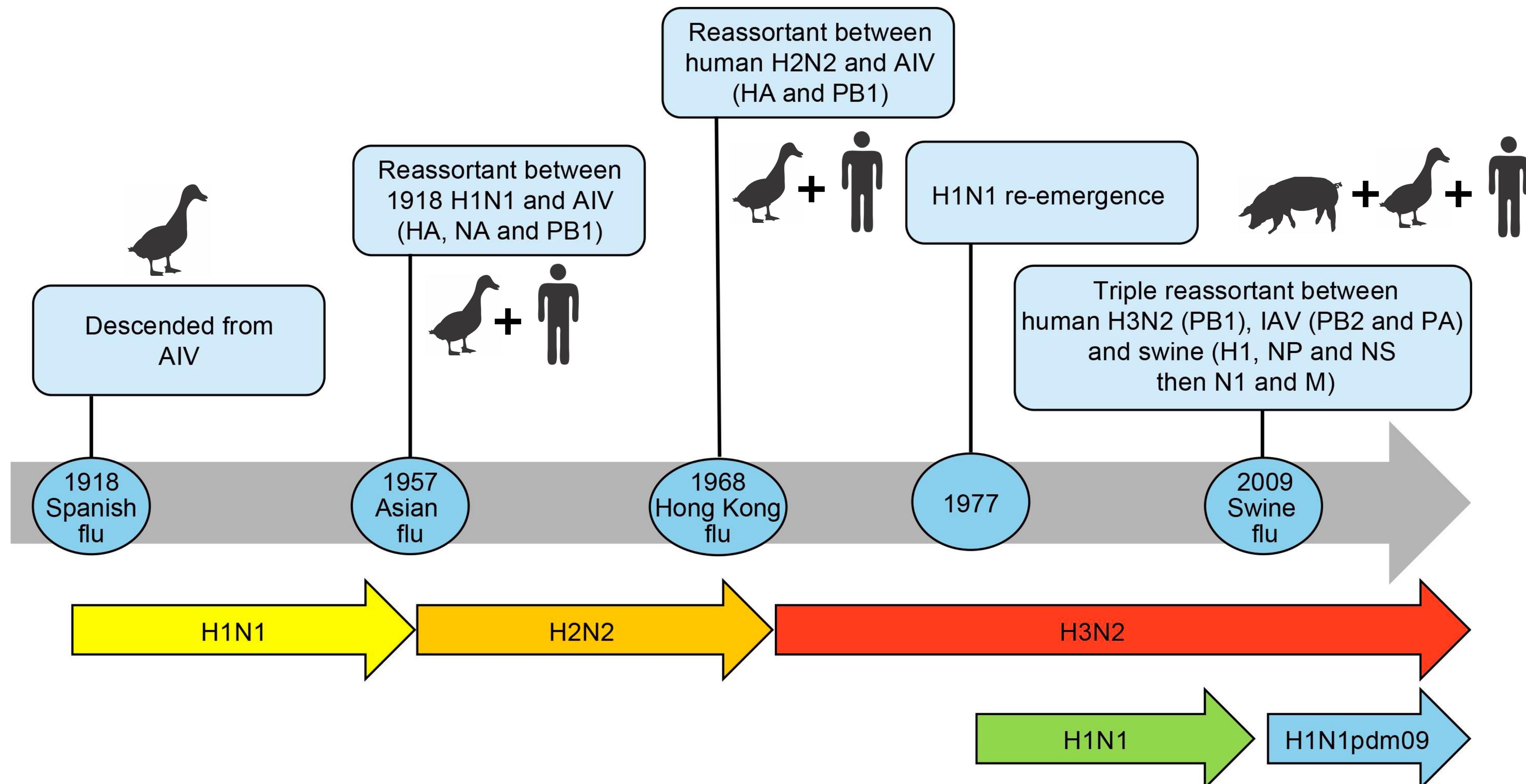


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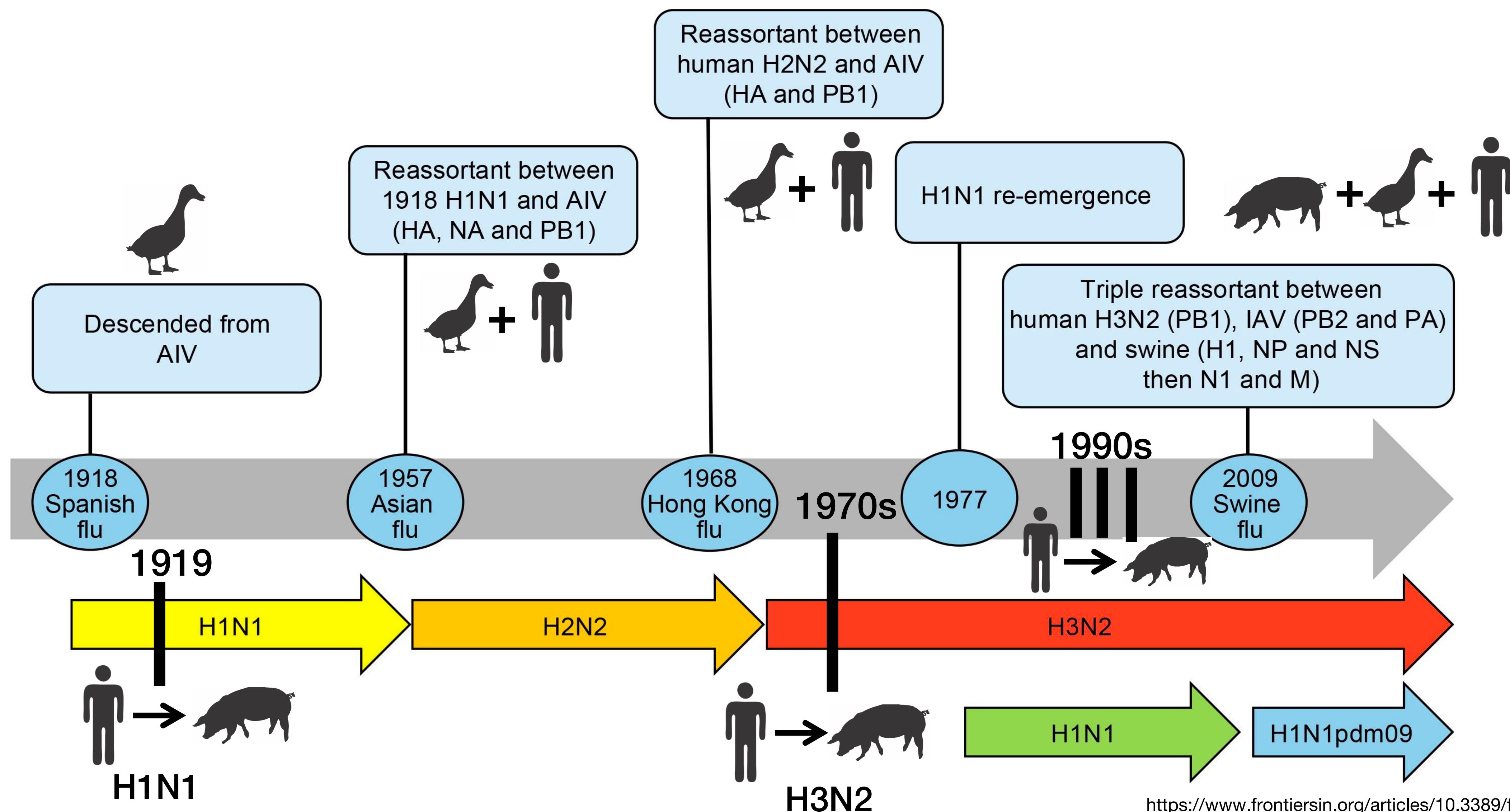
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Influenza pandemics and panzootics arise from reassortment and introduction into new animal populations



Humans recurrently introduce novel H1N1 and H3N2 viruses into swine populations



Humans and pigs transfer viruses between each other repeatedly, every year



Recap of influenza host ecology

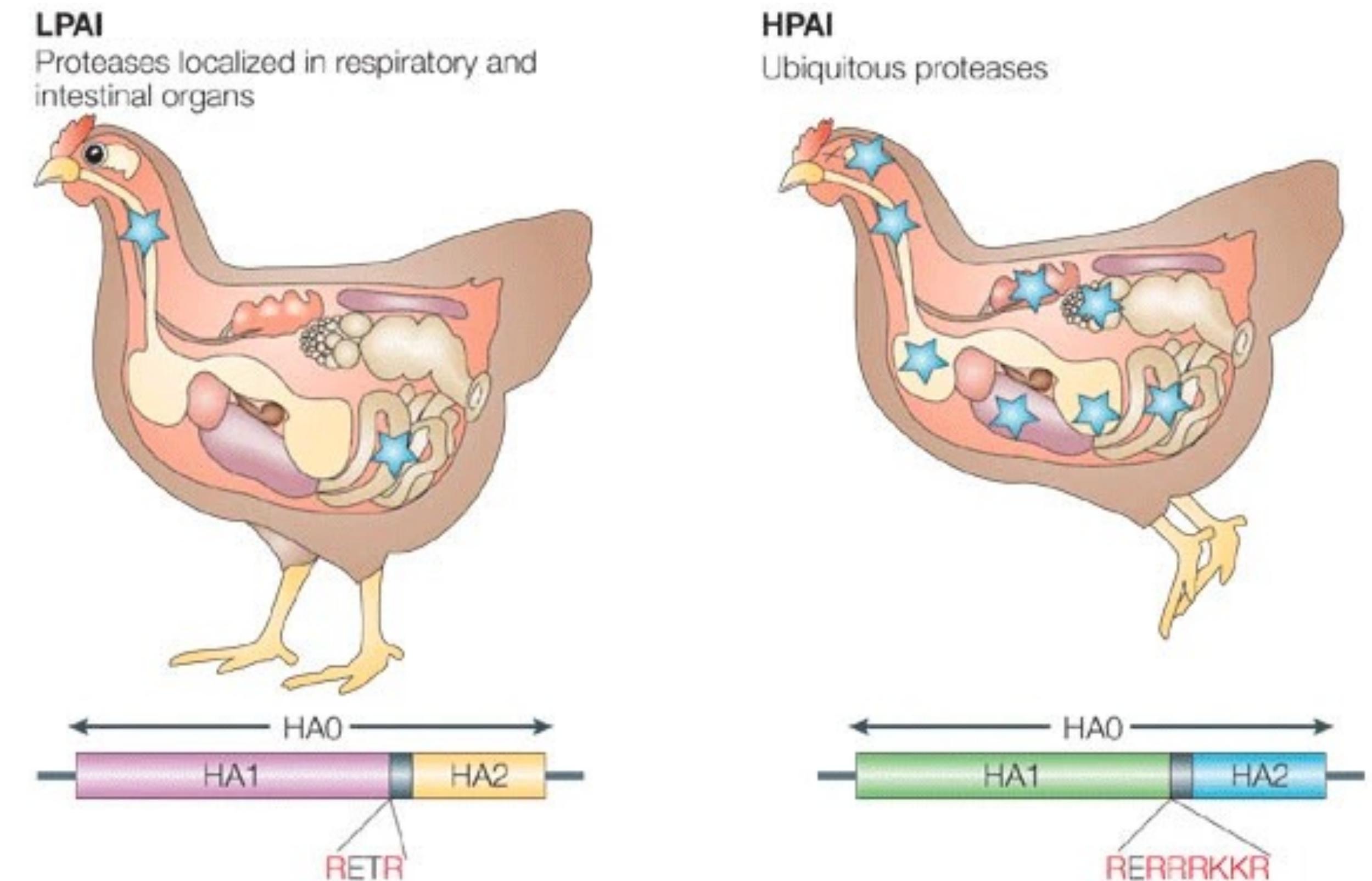
1. Influenza A's natural reservoir is wild, aquatic birds (Anseriformes)
2. Influenza viruses repeatedly and frequently spill over into new host species, and have embedded and circulate endemically in dogs, horses, pigs, humans, and poultry.
3. Influenza viruses can shuffle gene segments via reassortment, which can lead to the generation of new subtypes against which some host species do not have prior immunity. This process is called **antigenic shift** and can cause pandemics.
4. There have been 4 natural human influenza pandemics since 1900, and all were caused by viruses from other host species. Generally, antigenically new viruses from animals cause a pandemic, and then continue to circulate as seasonal epidemics in humans.
5. **Antigenic drift**, the gradual accumulation of mutations that escape population immunity, complicates vaccination strategies, and necessitates yearly updates in the human influenza vaccine.
6. Pigs are sometimes referred to as “mixing vessels” for flu because they can be infected by a wide variety of influenza viruses, facilitating reassortment. Humans have repeatedly infected pigs with our viruses.

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1. Influenza structure and life cycle
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3. **Emerging public health issues in influenza**
 - **Global spread of high path avian influenza**
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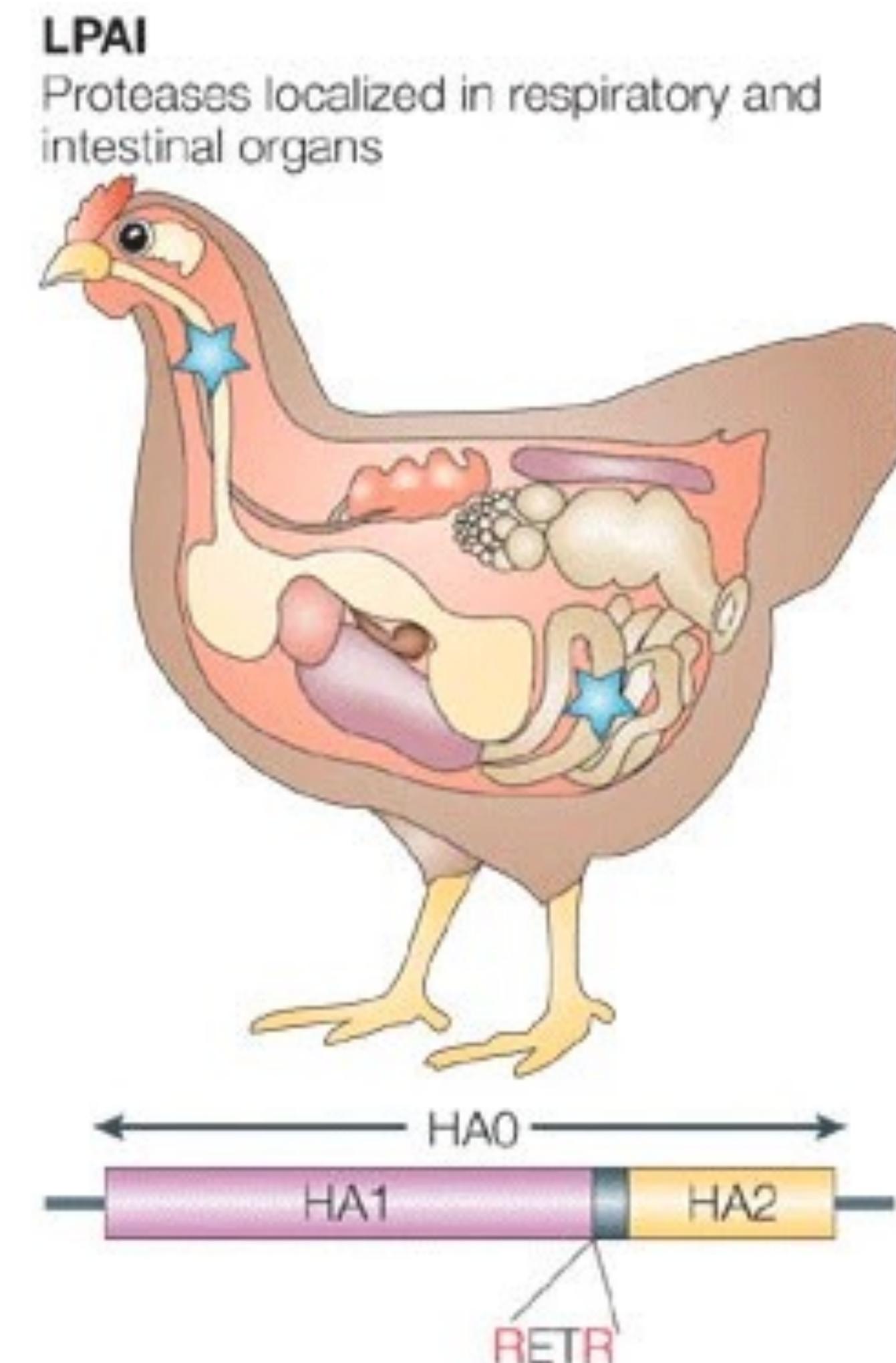
Highly pathogenic avian influenza (HPAI)

- High pathogenicity is defined by pathogenicity in experimentally infected chickens
- Primarily occurs in H5 and H7 viruses
- First evolved in H5N1 viruses in 1996 in poultry in China

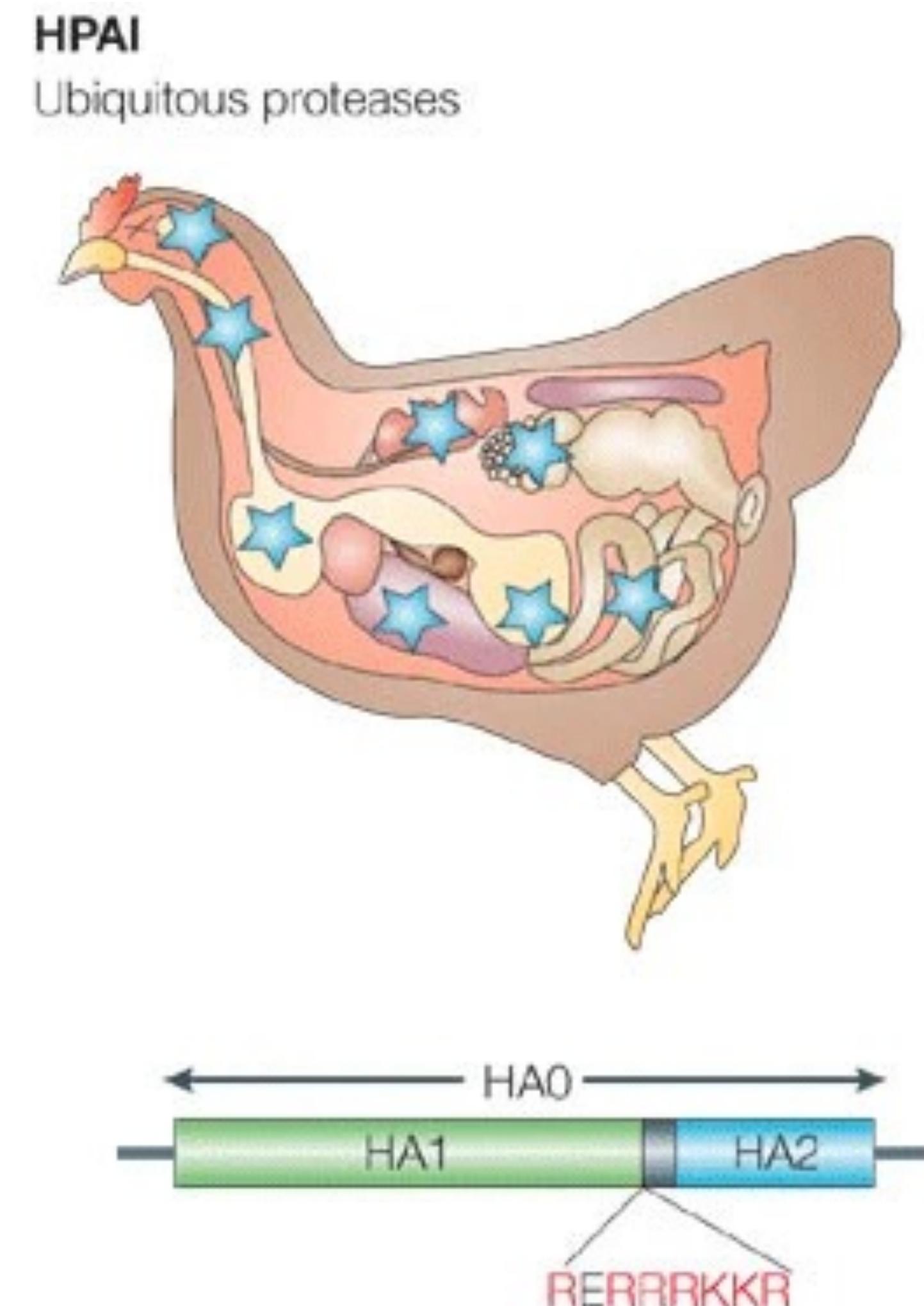


High pathogenicity strains are generated when a polybasic cleavage site is inserted into HA, allowing cleavage by ubiquitous proteases and systemic spread in the body

1. Low pathogenicity avian influenza (LPAI) strains contain a cleavage site targeted by trypsin-like proteases, which are localized to respiratory and intestinal organs

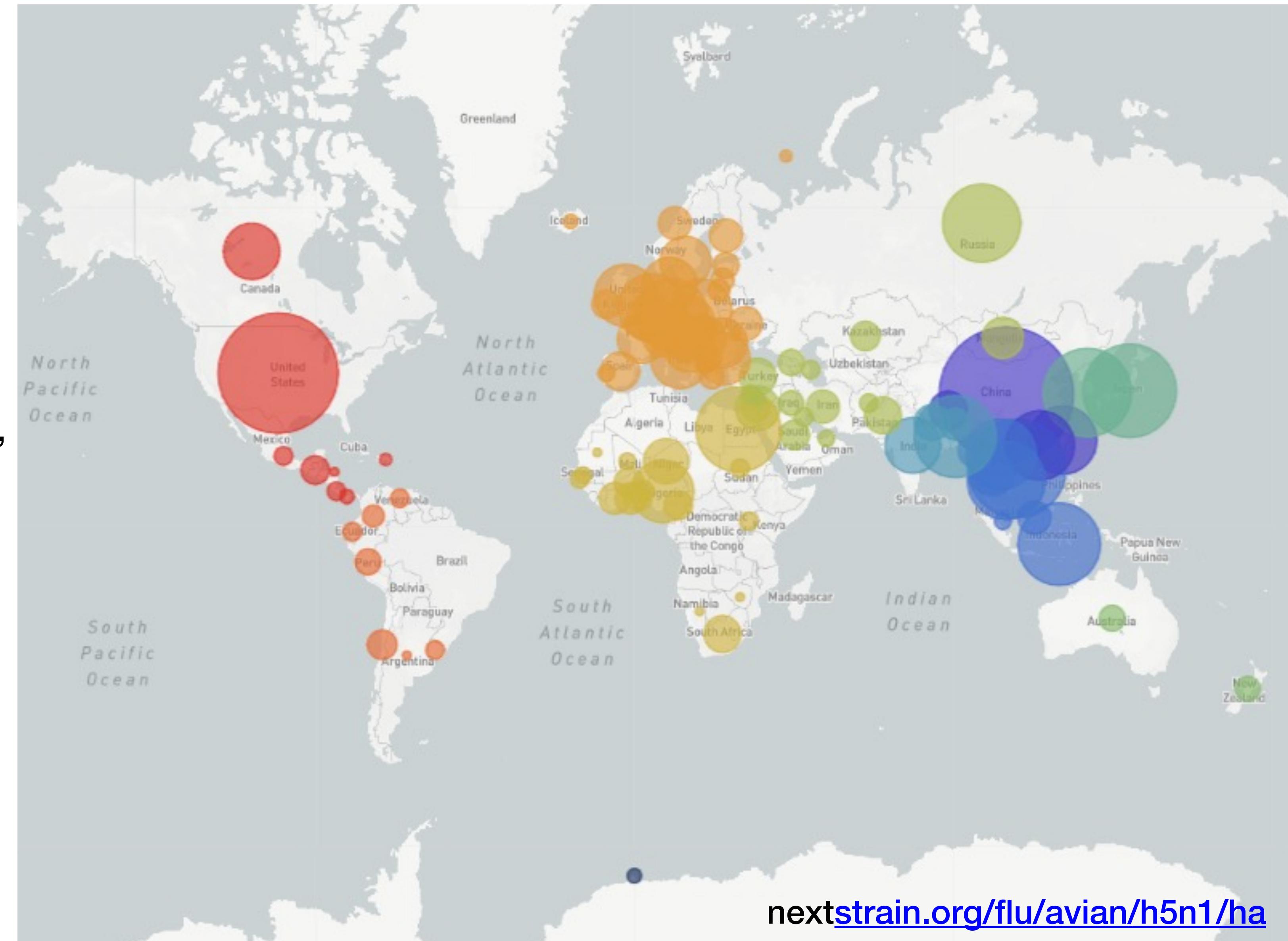


2. High-pathogenicity avian influenza viruses contain a polybasic cleavage site that is targeted by furin proteases, which are ubiquitous. This causes systemic spread.



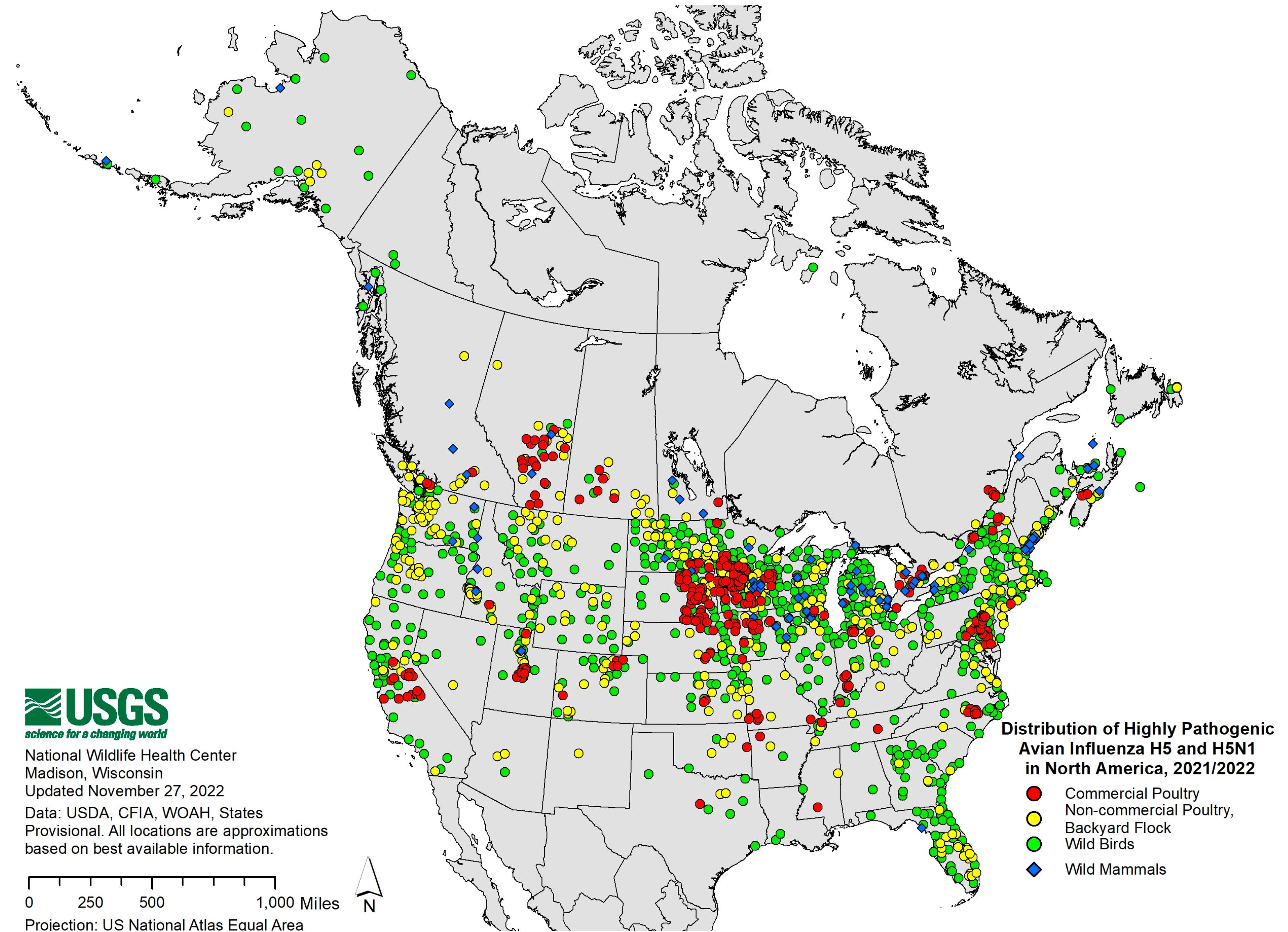
Highly pathogenic H5Nx avian influenza has spread globally via wild bird migration and poultry trade networks

1. Highly pathogenic H5Nx viruses first emerged in domestic birds in China, and became endemic in poultry in Asia via poultry trade.
2. In 2005, these viruses were reintroduced into wild birds in China, which spread H5Nx into Africa, Europe, and Southeast Asia and established endemic, circulating lineages in domestic birds.
3. Wild birds spread H5Nx long distances, while poultry disseminate across local geographic space.



In late 2021, clade 2.3.4.4b viruses caused an panzootic in Europe and the Americas that is still ongoing

- Panzootic = animal pandemic
- Began December 2021 in Canada, then spread throughout North America, to South America, and recently to Antarctica
- ~60 million domestic birds culled in US
- Hundreds of thousands of wild birds died



High path avian influenza has impacted wildlife, including non-canonical bird species and wild mammals

AUGUST 29, 2023 | 6 MIN READ

Endangered California Condors Get Bird Flu Vaccine

After avian influenza killed 21 endangered California Condors, government officials are testing a vaccine that could protect the massive scavengers from infection

BY MEGHAN BARTELS

August 9, 2023



An unprecedented flu strain is attacking hundreds of animal species. Humans could be next.

An avian flu panzootic — a pandemic among animals — has struck hundreds of bird and mammal species, including elephant seals.



September 27, 2023

Bird flu kills hundreds of flamingos in Argentina

27th November 2023, 12:00 EST

January 15, 2024



High path avian influenza has dramatically impacted agriculture

Bird Flu Outbreak Puts Mink Farms Back in the Spotlight

A new variant of avian influenza appears capable of spreading among mammals, highlighting the need for more proactive surveillance, experts said.

February 8, 2023



Give this article 73

Philippines bans poultry imports from California, Ohio to prevent bird flu spread

Reuters

January 16, 2024 11:20 PM EST · Updated 3 days ago



January 16th, 2024

Open questions in high path avian influenza

1. What will be the long-term impact of endemic establishment in American wild birds?
2. Which public health interventions (targeted/broad vaccination, culling, biosecurity updates), or combination of interventions, will best mitigate adverse outcomes for wildlife and agriculture?
3. What types of surveillance and risk assessments need to be done to reduce the risk of spillover into humans or other mammals?

Canine and equine influenza

FEBRUARY 24, 2023

- Canine influenza periodically causes outbreaks among shelter animals
- H3N8: circulated 2002-2016, derived from equine lineage
- H3N2: circulated 2004-present, direct transmission from birds in Asia
- Vaccine is available and can be used in outbreaks

How to keep dogs safe from canine influenza, which is on the rise in Philadelphia

Pet owners should not share toys or bowls or take trips to busy parks, Dr. Amber Karwacki of Callowhill Heart + Paw suggests



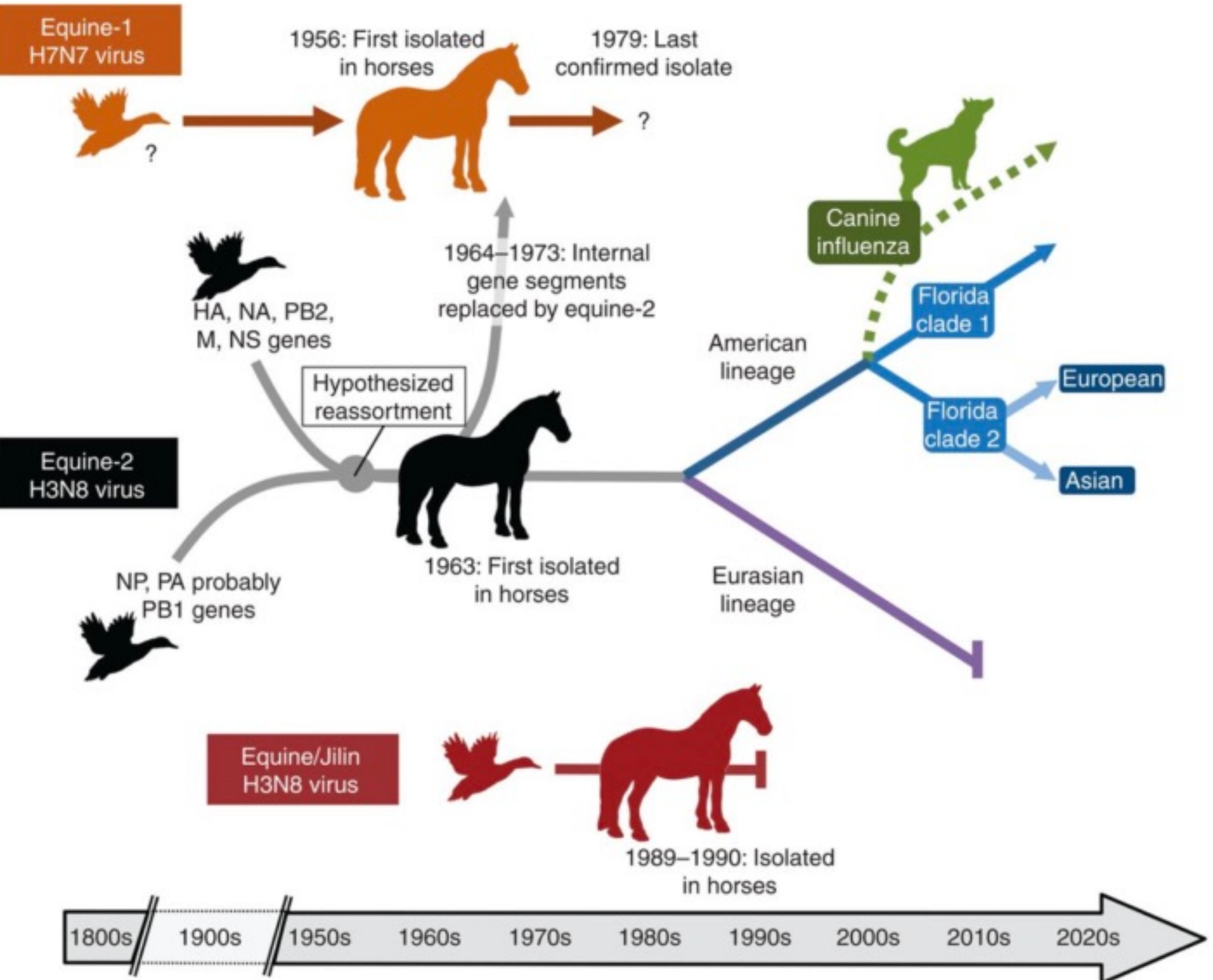
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PETS Dogs



Multiple reassortment and cross-species transmission events led to equine and canine influenza viruses



Recap of emerging issues in influenza

1. High-pathogenicity H5Nx avian influenza viruses cause recurrent problems for agriculture, wild animal health, and human health.
2. The 2021 panzootic poses new challenges for avian influenza ecology and control.
3. Equine influenza (H3N8) and canine influenza (H3N2) continue to circulate and cause periodic outbreaks.

Final take-home messages:

1. Influenza viruses are -ssRNA viruses with a segmented genome.
2. HA binds to sialic acid to enter the host cell, and escapes the endosome during acidification due to HA and M2 activity. Transcription occurs via cap snatching, and vRNP production proceeds through a complementary + sense intermediate. NA cleaves sialic acid receptor during viral exit from the host cell.
3. Influenza A viruses naturally circulate in wild, aquatic birds, but frequently jump between hosts. Pigs, dogs, horses, humans, and poultry all sustain endemic influenza circulation. Pigs are often cited as “mixing vessels” because they can support replication of avian and human strains.
4. Reassortment shuffles genetic diversity, and can generate new viral subtypes that can cause pandemics via antigenic shift.
5. HPAI evolves when a polybasic cleavage site evolves in HA. HPAI of the H5 subtype is currently causing a panzootic that impacts wildlife and agriculture.
6. Influenza evolves yearly via antigenic drift, which necessitates frequent vaccine strain updates.