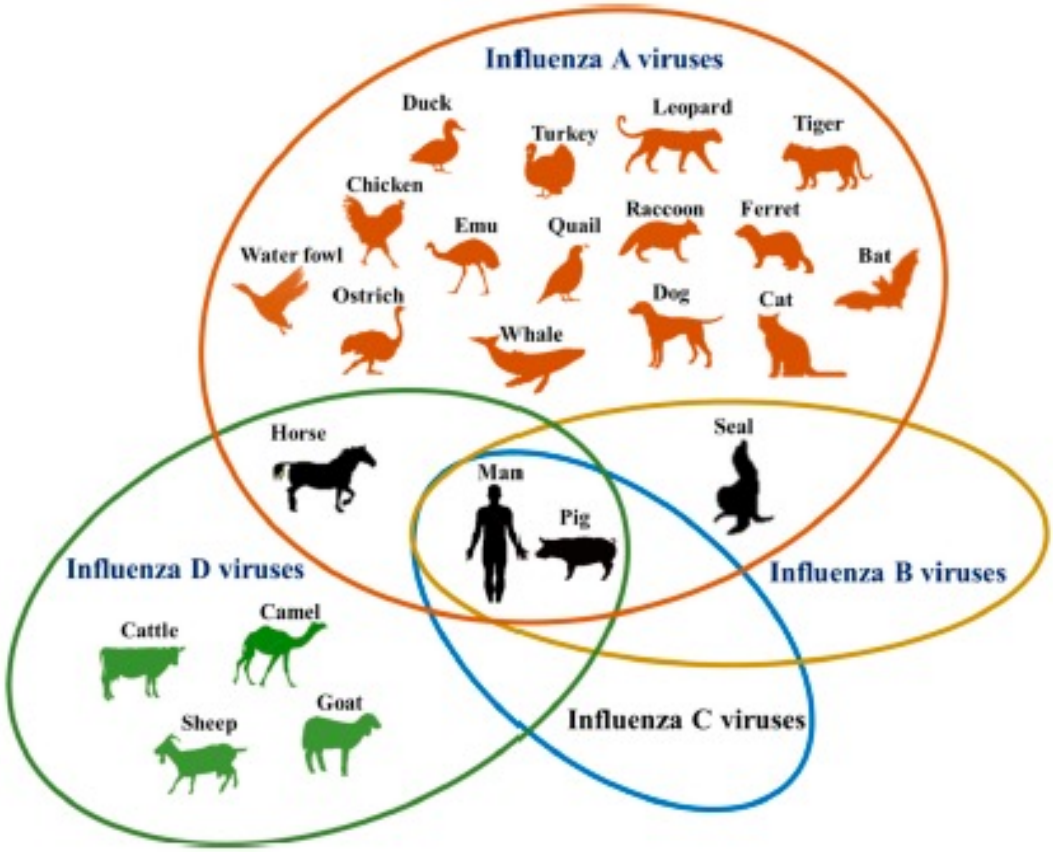
Orthomyxoviridae

Table 1. Revised classification of *Orthomyxoviridae* (ICTV 2017).

Genus	Species	Genomic Segments
Alphainfluenzavirus	Influenza A virus	8
Betainfluenzavirus	Influenza B virus	8
Deltainfluenzavirus	Influenza D virus	7
Gammainfluenzavirus	Influenza C virus	7
Isavirus	Salmon isavirus	8
Quaranjavirus	Johnston Atoll quaranjavirus	6
	Quaranfil quaranjavirus	
Thogotovirus	Dhori thogotovirus	6
	Thogoto thogotovirus	

Host range of influenza viruses by species



Common hosts of more than one species are encompassed in overlapping ovals. Of the numerous hosts which support influenza virus infection, only four (horse, seal, man and pig) are known to be susceptible to more than one species.

Vet. Sci.2018,5, 71; doi:10.3390/vetsci5030071 www.mdpi.com/journal/vetsci

Influenza virus host range

•**Influenza A viruses** infect a wide variety of mammals **and** birds. IAV is the main human pathogen, associated with epidemics and pandemics. Many different subtypes exist according to the type of haemagglutinin (HA) and neuraminidase (NA) expressed. Pigs and birds are believed to be particularly important reservoirs, generating pools of genetically/antigenically diverse viruses, which can be transferred back to the human population via close contact between humans and animals.

•**Influenza B viruses** infect mammals only and cause disease, but generally not as severe as A types. Unlike influenza A viruses, influenza B viruses do not have distinguishable subtypes.

•**Influenza C viruses** also infect mammals only, but rarely cause disease. They are genetically and morphologically distinct from A and B types.

•**Influenza D viruses** The virus was first isolated as an influenza C-like virus from pigs with respiratory illness in Oklahoma, USA, in 2011. The virus was subsequently classified as influenza D virus (IDV). While the precise role of IDVs in clinical disease in animals is not yet fully investigated, their role in causing respiratory infections in cattle has been implied.

Influenza virus Transmission

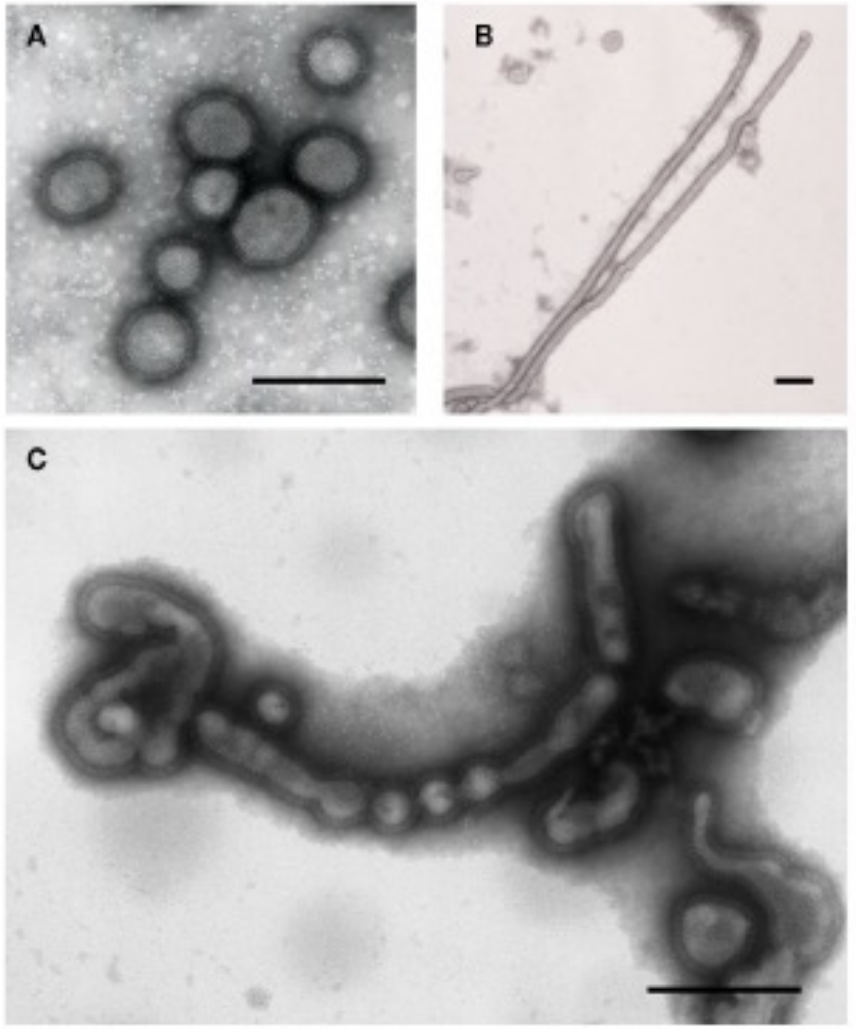
- AEROSOL
  - 100,000 - 1,000,000 VIRIONS/DROPPLET
- INCUBATION 18-72 HOURS

RECOVERY

- INTERFERON – SIDE EFFECTS INCLUDE:
  - FEVER, MYALGIA, MALAISE, FATIGUE
- CELL-MEDIATED IMMUNE RESPONSE
- TISSUE RIPAIR:
  - CAN TAKE LONGER

Morphology of influenza virions

IAV virions show spherical or filamentous shapes of about 100nm in diameter and occasionally irregular morphology, which exemplifies the pleomorphic nature of these virions..



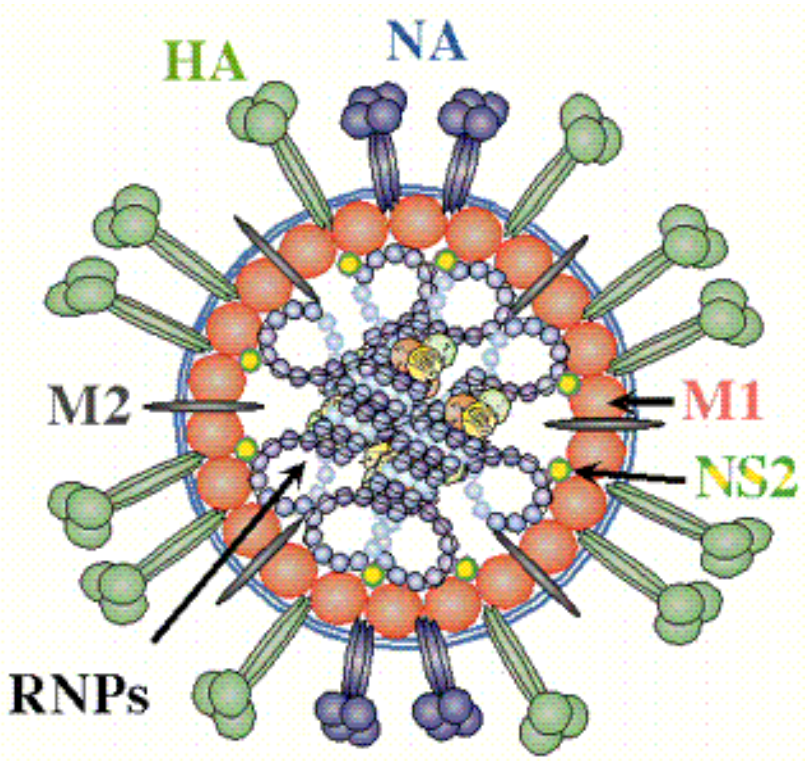
Structure and components

The outer layer of the lipid envelope is spiked with multiple copies of **Hemagglutinin** (**HA**) trimers and **Neuraminidase (NA)** tetramers.

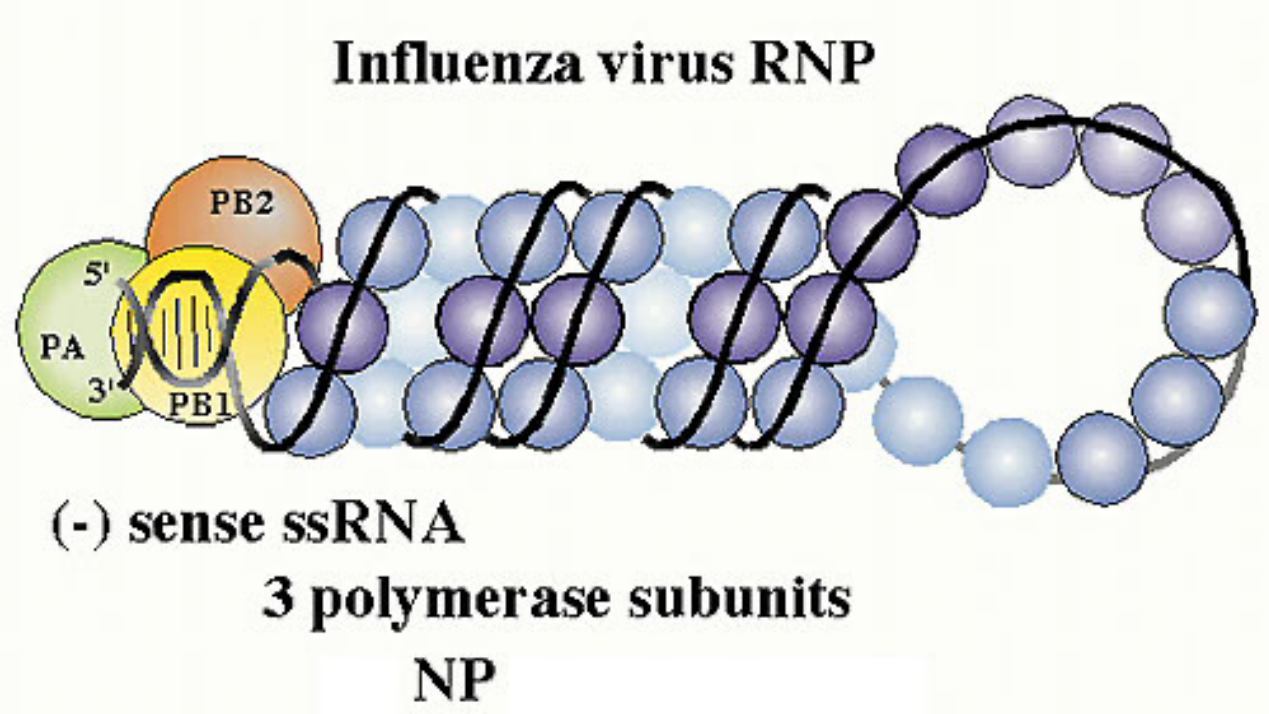
A small number of **M2** proteins forms the ion channels across the envelope

The **M1** (**Matrix protein**) molecules keep **vRNPs** attached to the inner layer

NS2, nuclear export protein (NEP)

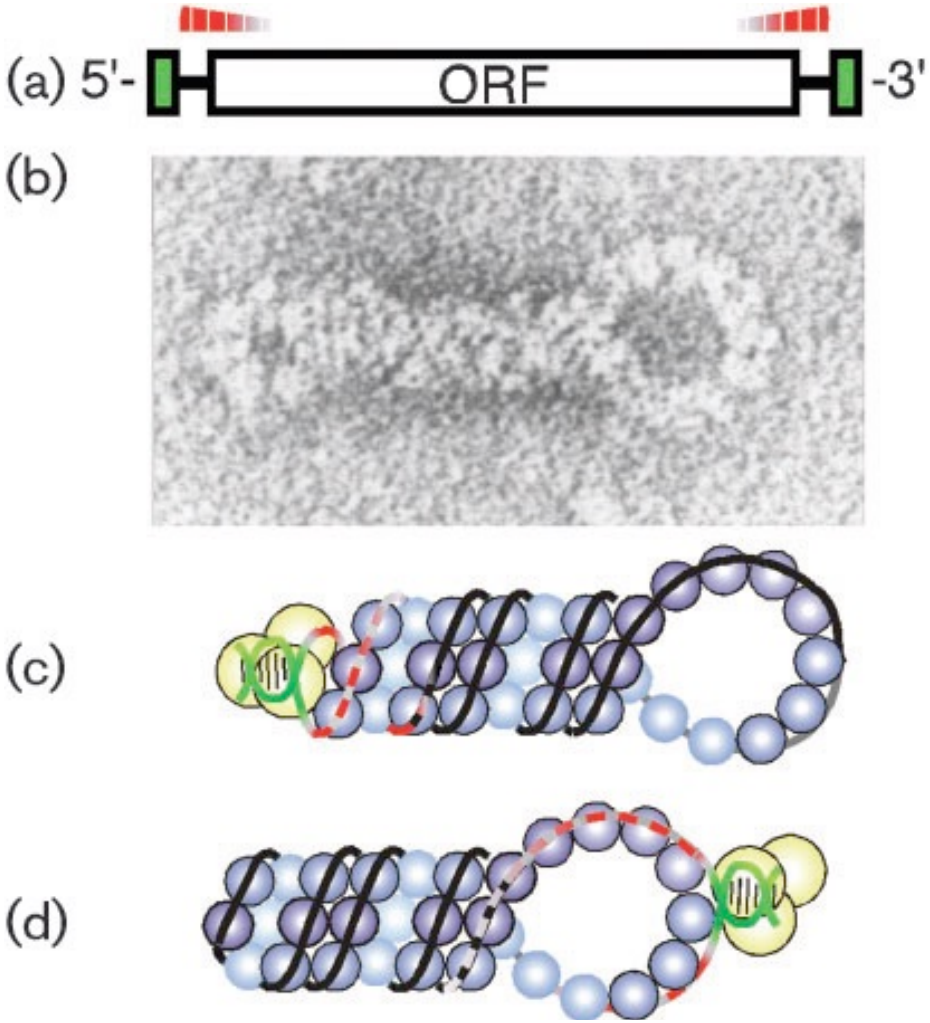


GENOME (-) ssRNA – 8 SEGMENTS



v-**RNP:** RNA + nucleoprotein (**NP).** The components of the **RNA-dependent RNA polymerase complex (PB1, PB2 and PA)** associates to each segment

GENOME (-) ssRNA – 8 SEGMENTS



A large ORF (open box) is flanked by short UTRs containing terminal promoter sequences (green boxes) that form the polymerase binding site, they are essentially identical in all segments and show partial complementarity. Specific packaging signals (red wedges) overlap the UTRs and terminal coding regions and are apparently discontinuous.

Journal of General Virology (2010), 91, 313–328

RNAs in infected cells

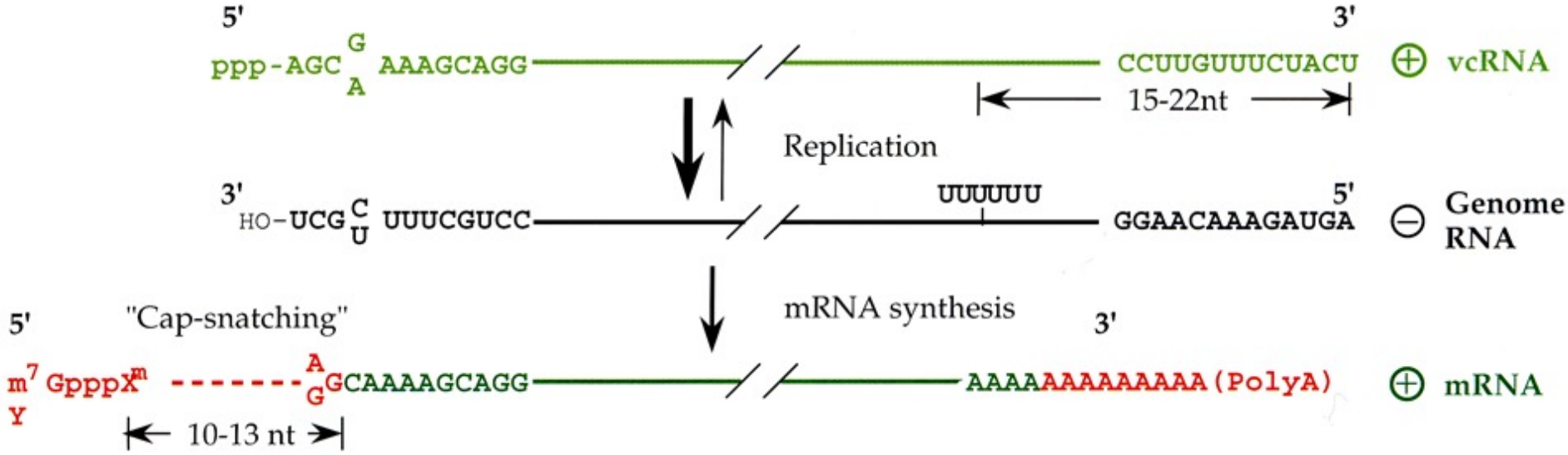
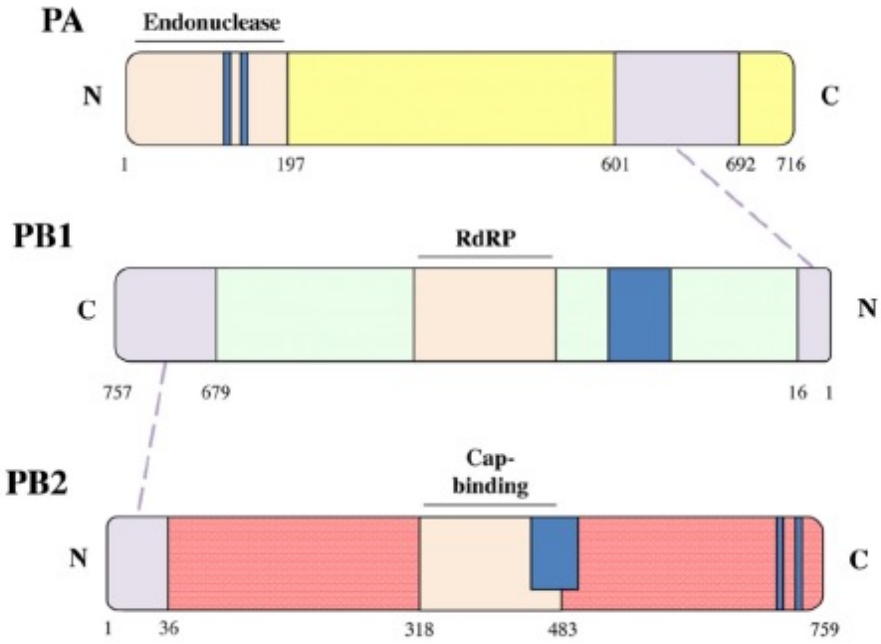


FIGURE 4.13 Relationship between genome RNAs, mRNAs, and vcRNAs of influenza virus. Synthesis of mRNAs in the cell nucleus requires a primer of 10–13 nucleotides derived from cellular pre-mRNAs by "cap-snatching," and mRNAs terminate with a poly(A) tail. Those portions of the mRNA that are not complementary to the genome RNA are shown in red. In contrast, vcRNAs are exact complements of the genomic minus strands. [Adapted from Strauss and Strauss (1997).]

Influenza A virus genome segments

Segment	Size (nt)	Protein	Function
1	2341	PB2	Cap-binding (transcription)
2	2341	PB1 PB1-F2 N40	RNA-dependent RNA polymerase (transcription and replication) Apoptosis, inflammation, enhances polymerase activity Unknown
3	2233	PA PA-X	Endonuclease Shutoff of host protein expression at late stage of infection
4	1778	HA	Hemagglutinin, receptor binding, fusion
5	1565	NP	Nucleoprotein, cytoplasm-nucleus translocation of v-RNP
6	1413	NA	Neuraminidase: virion release (main)
7	1027	M1 e M2	M1= matrix M2= ion channel
8	890	NS1 e NS2	NS1 = host function interference, absent inside the virion NS2 or NEP = v-RNP nuclear export

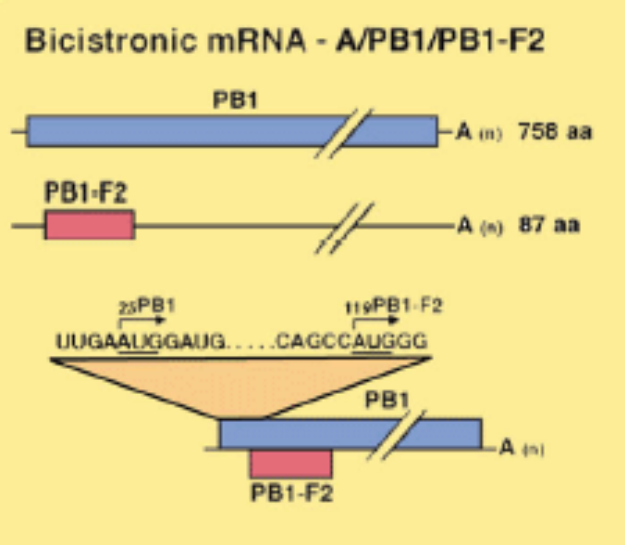
IAV polymerase complex



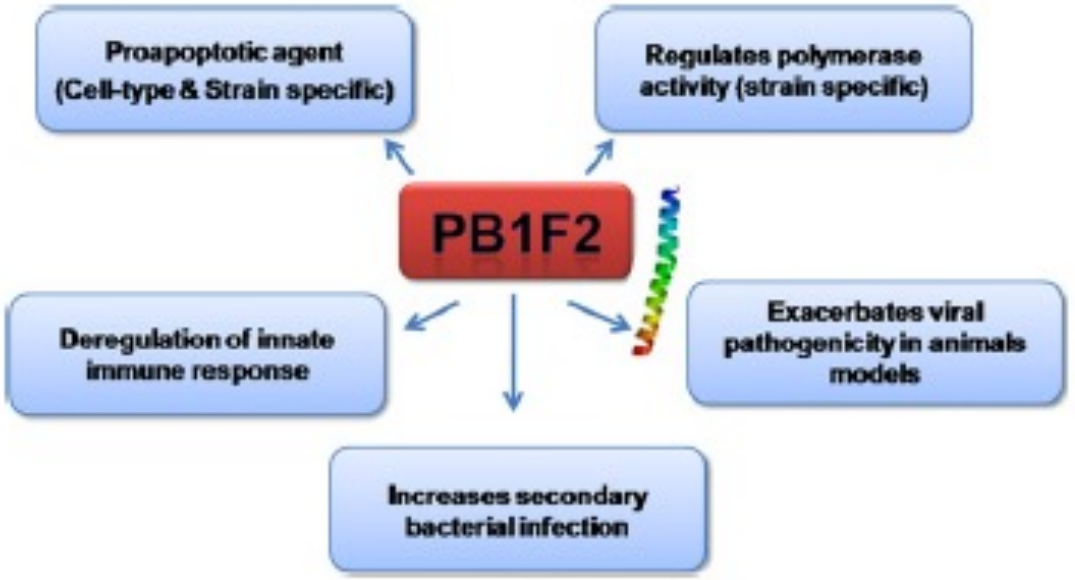
The interaction domains between proteins, as well as the functional domains are indicated. NLS of each protein are depicted in blue

Virus Research 209 (2015) 23–38

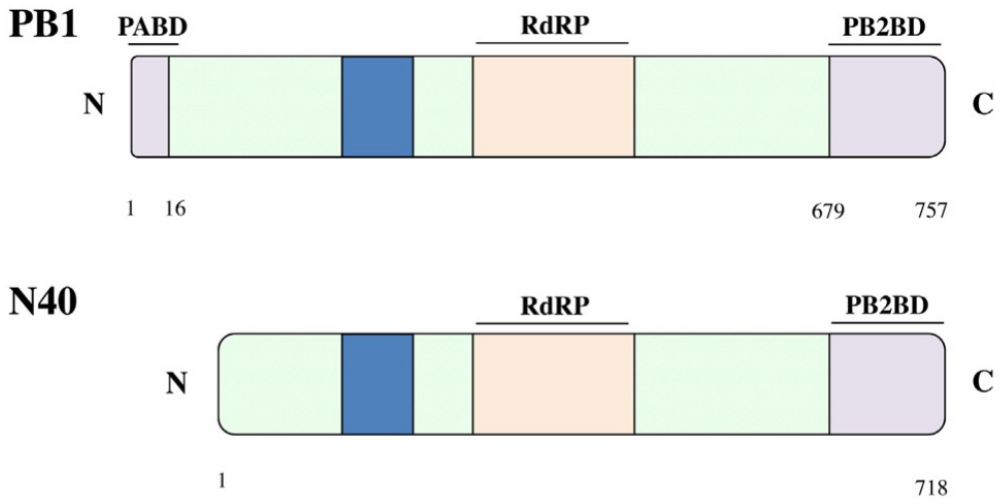
Genome segment 2 PB1-F2



Here we see the two open reading frames of the PB1 gene segment of Influenza A Virus. The red segment corresponds to the alternate reading frame that encodes the PB1-F2 protein whose start site is 120 bp downstream of the PB1 polymerase gene. This figure was adapted form Lamb et al. 2001.



Genome segment 2 N40



A leaky ribosomal scanning process is involved in N40 expression due to the presence of a strong Kozak translation initiation context in the 5th in frame AUG codon of PB1 gene. This initiation codon is located 115 nucleotides downstream from the first AUG in PB1 mRNA and thus, the N40 protein is an amino-terminally truncated version of the PB1 protein that lacks the first 39 amino acids, residues where the PA binding domain is located.

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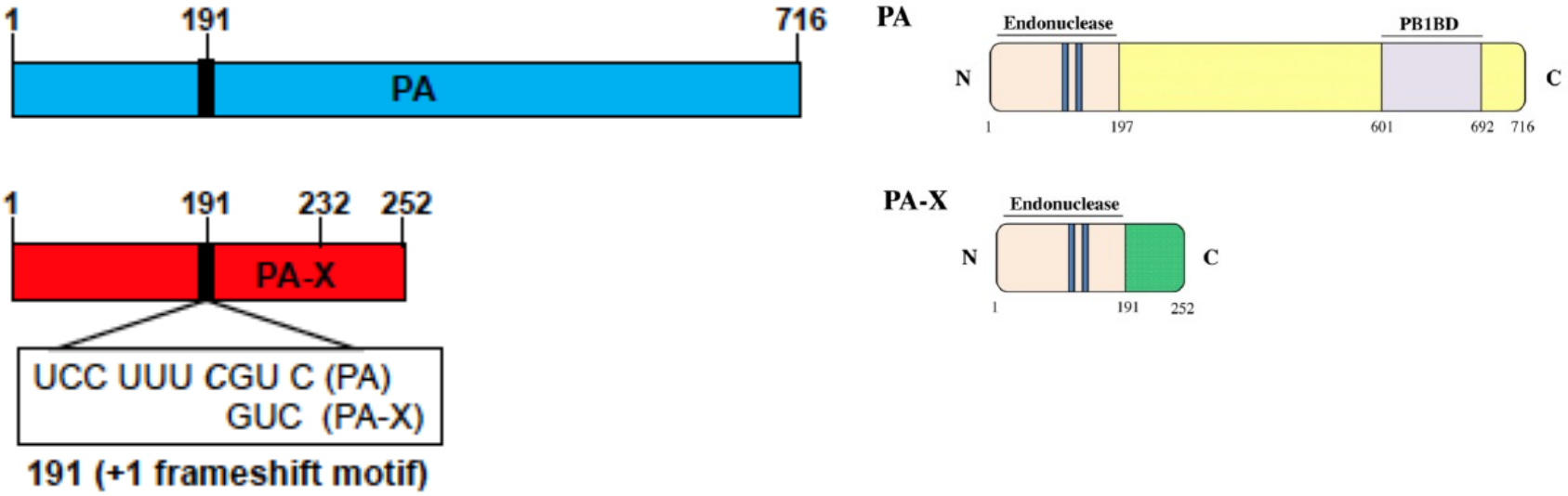
Genome segment 3 PA-X



A schematic representation of the IAV PA viral segment and the PA and PA-X open reading frames (ORFs). Blue and red boxes indicate the ORF for PA and PA-X, respectively. The +1 frameshift motif (UCC UUU CGU C) at position 191 is indicated. Bold and italics in the frameshift motif (C nucleotide) indicate that the nucleotide C is not read during PA-X translation. PA-X proteins containing 232 or 252 amino acids if the C-terminal region has a 41 or 61 amino acid extension, respectively, are indicated. PA-X selectively degrades host RNA polymerase II (Pol II)-transcribed mRNAs and non-coding RNAs in the nucleus of infected cells, while sparing the products of polymerases I and III.

Viruses 2018, 10, 708

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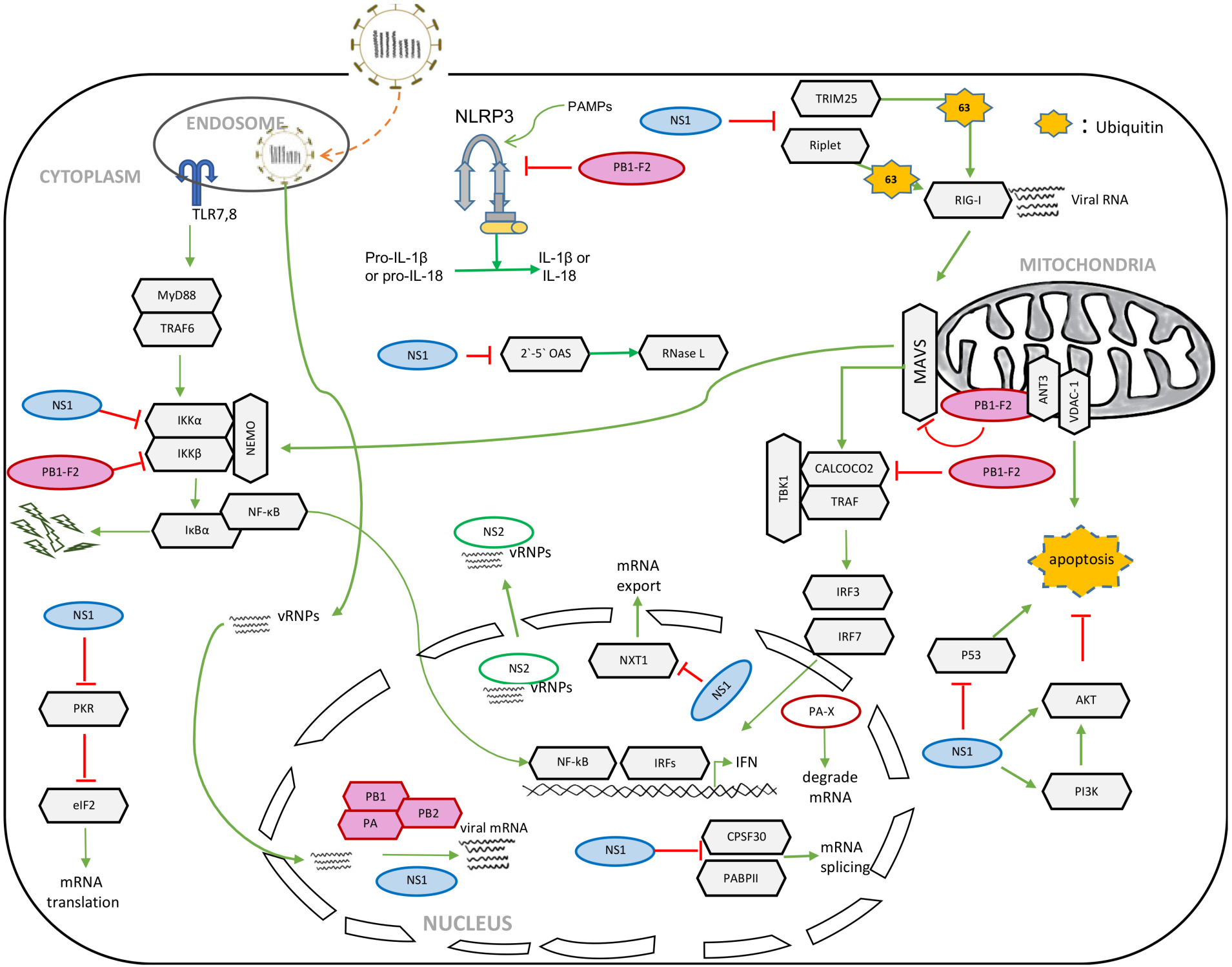
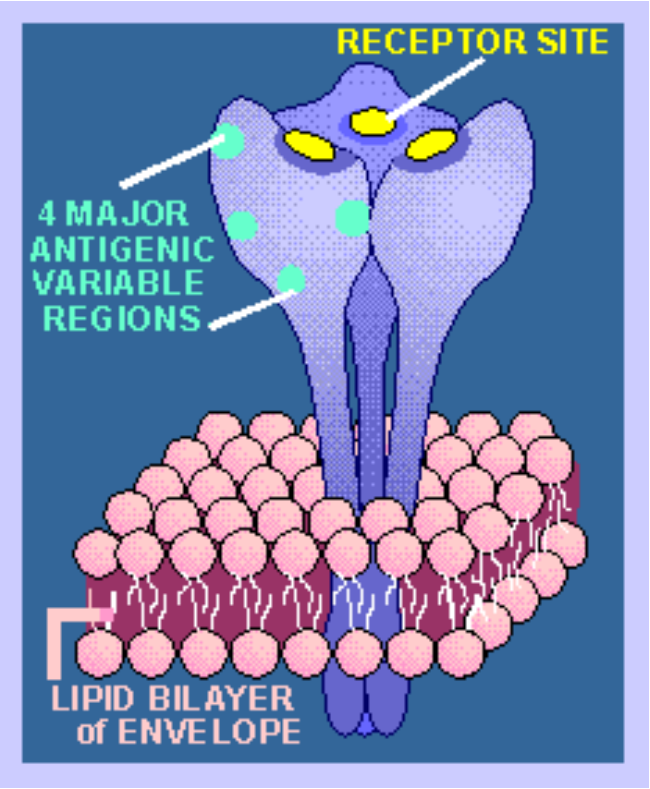
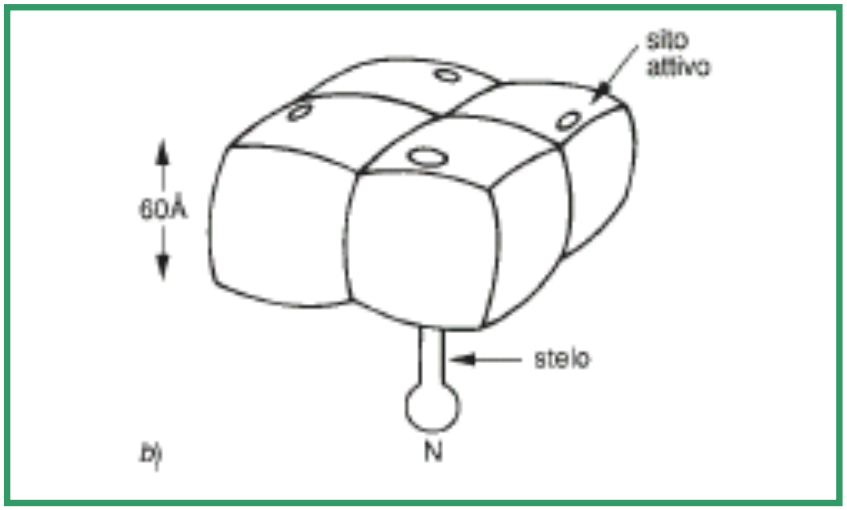

ENDOSOME
CYTOPLASM
TLR7,8
MyD88
TRAF6
NS1
IKKα
IKKβ
NEMO
PB1-F2
IκBα
NF-κB
NLRP3
PAMPs
NS1
PB1-F2
Pro-IL-1β
or pro-IL-18
IL-1β or
IL-18
TRIM25
63
Riplet
63
: Ubiquitin
RIG-I
Viral RNA
MITOCHONDRIA
MAVS
ANT3
VDAC-1
PB1-F2
NS1
2`-5` OAS
RNase L
TBK1
CALCOCO2
TRAF
PB1-F2
IRF3
IRF7
apoptosis
P53
AKT
PI3K
NS1
NS2
vRNPs
mRNA
export
NXT1
NS1
NS2
vRNPs
vRNPs
NS1
PKR
eIF2
mRNA
translation
PA-X
degrade
mRNA
NF-kB
IRFs
IFN
PB1
PB2
PA
viral mRNA
NS1
NS1
CPSF30
PABPII
mRNA
splicing
NUCLEUS



On the HA head, the major antigenic variable regions localize close to the receptor binding site



Antigenic variable epitopes characterize also neuraminidase (NA).

Genome segment 7

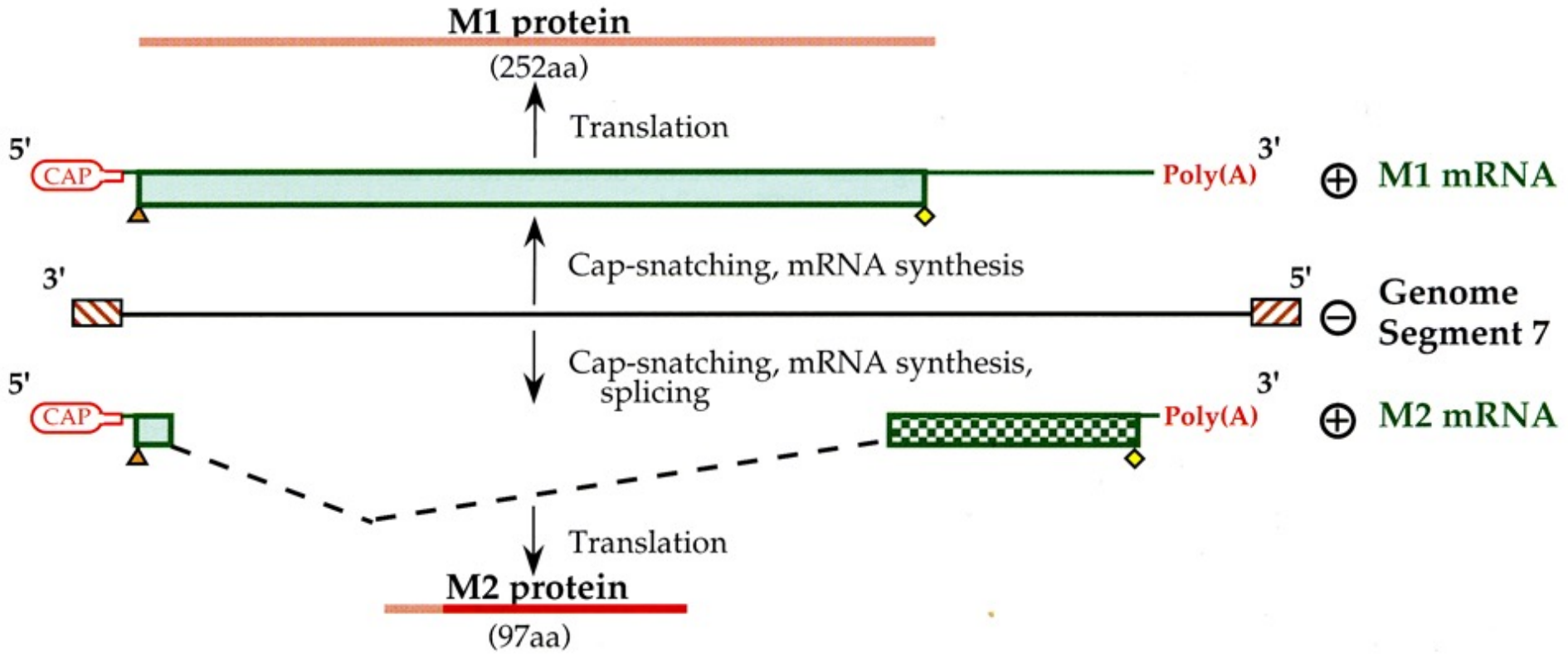
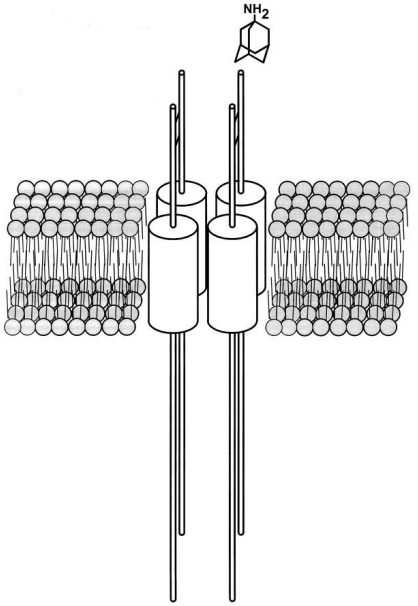


FIGURE 4.14 Synthesis of two mRNAs for the M1 and M2 proteins from gene segment 7 of influenza A. M1 RNA is translated from ORF1 (open box). M2 RNA starts identically, but after the splice it is translated in ORF2 (checked box). Both proteins are found in infected cells. The AUG initiation codon is shown as a triangle; termination codons are shown as filled diamonds. Patterned boxes at the end of the genome RNA are self-complementary sequences not present in the mRNAs that could form panhandles.

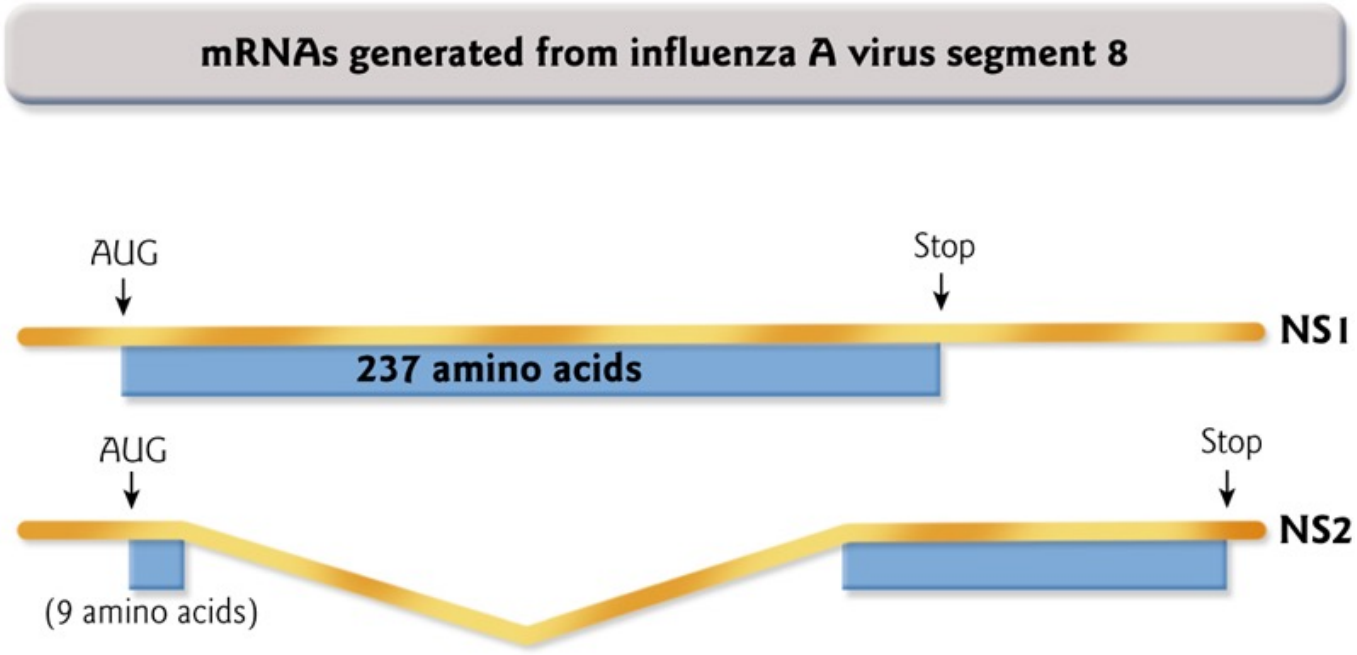
The M1 and M2 proteins

- The M1 matrix protein is located inside the lipid envelope and mediates the assembly and budding of the virus particle.
- The M2 protein forms active ion channels for proton transport. Ion channel activity is essential during the virus uncoating.
- These channels are specifically blocked by the antiviral drug amantadine hydrochloride.



The M2 protein forms homotetramers held together by disulfide bridges

Genome segment 8



The NS gene

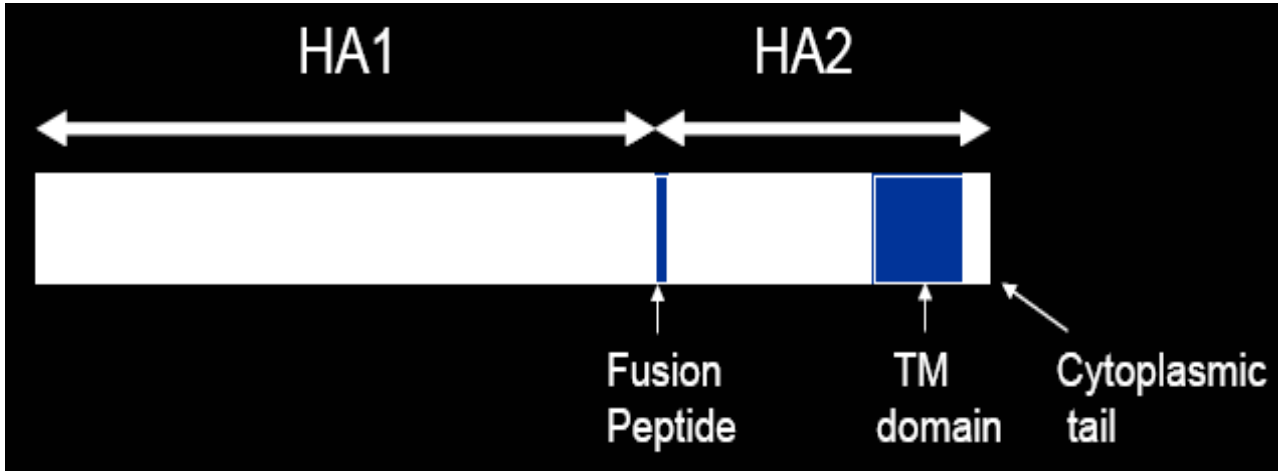
- The NS gene encodes two proteins, NS1 and NS2. NS1 is encoded by the genomic transcript, while NS2 is encoded by a spliced mRNA.
- NS1: anti-IFN activity, pre-mRNA accumulation in the nucleous
  - The NS1 protein of the influenza A virus i) binds to dsRNA, ii) prevents activation of PKR and OAS mediated by dsRNA, iii) prevents the synthesis of Interferon. All these activities have been mapped to NS1 N-terminal domain.
  - Through the interaction of the C-terminal portion with cellular proteins NS1 inhibits the polyadenylation of cellular mRNAs and their accumulation in the cytoplasm, increasing the pre-mRNA concentration in the nucleus and their availability for viral functions
- NS2 (NEP for Nuclear Export Protein): involved in the nuclear export of the vRNPs

LIFE CYCLE

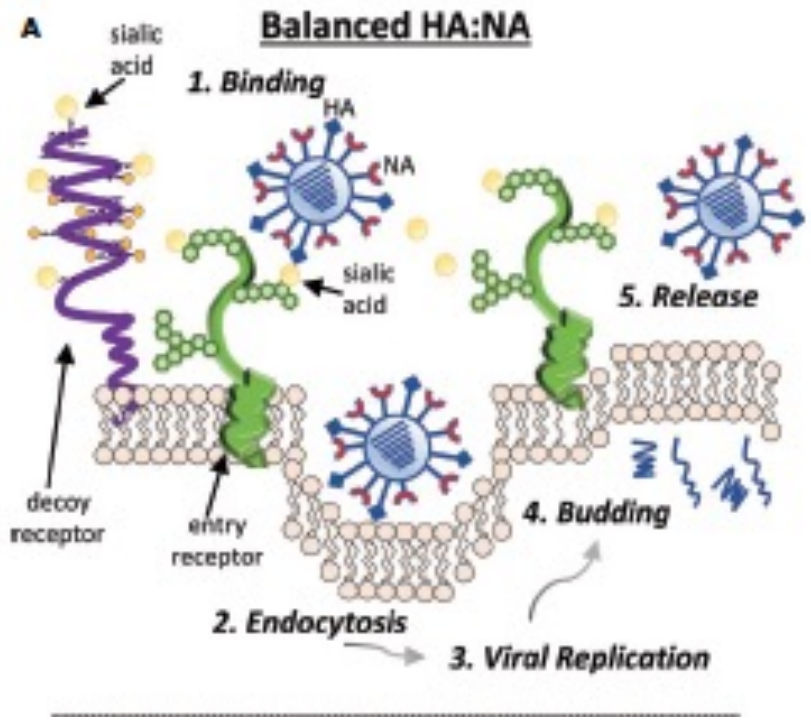
Among the RNA(-) viruses, the influenza A virus represents an exception, as the synthesis of the RNAs (during both transcription and replication) and the assembly of the vRNPs takes place inside the nucleus of the infected cell.

Life Cycle (attachment)

•The attachment of the virion to the host cell is mediated by the interaction of HA with glycoproteins containing sialic acid residues.
•HA is synthesized as a precursor (HA0), which is cleaved (trypsin-like enzymes or furin) into HA1 (responsible for interaction with the receptor) and HA2, which includes the trans-membrane domain and the fusion peptide (responsible for envelope fusion with endosomial membrane). The HA1 and HA2 fragments are held together by disulfide bridges.

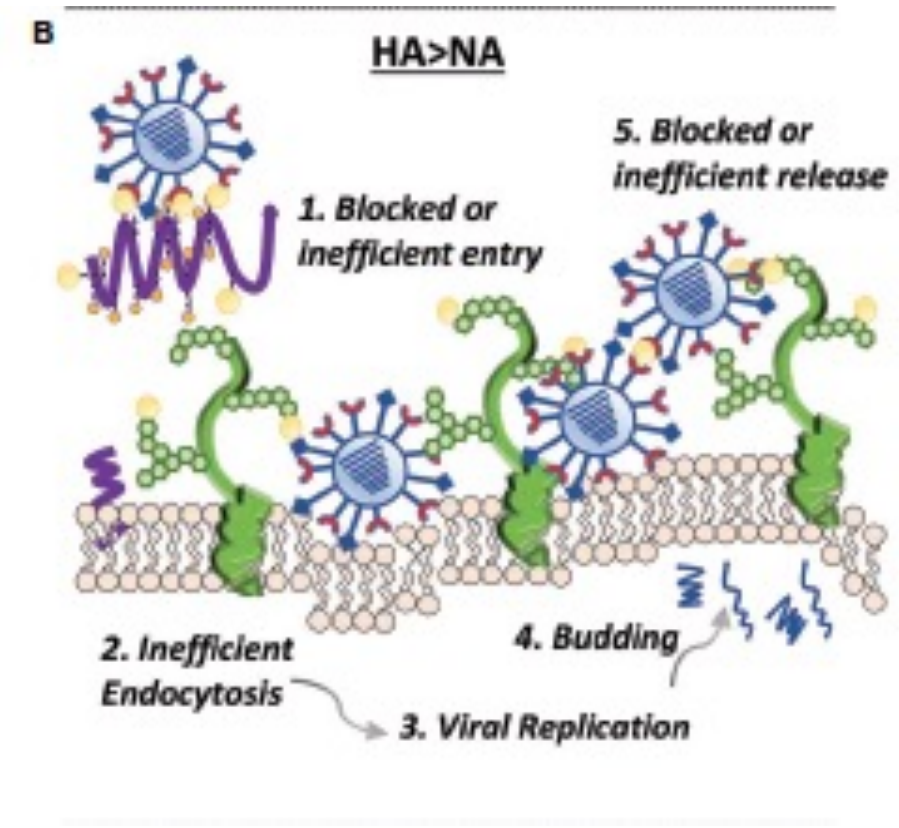


The relative activity of the HA and NA needs to be balanced to maintain the ability of the virion to efficiently infect and be released from cells (HA:NA=6:1)



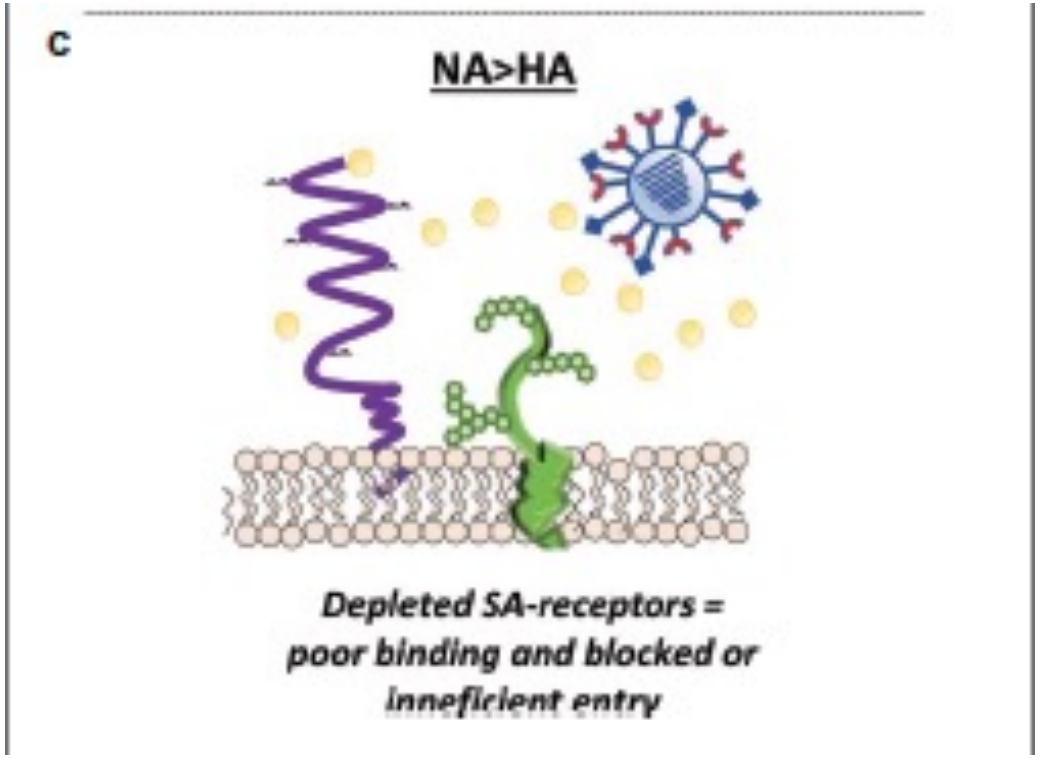
Efficient cleavage of sialic acids from decoy receptors (such as cell-surface mucins) by NA enables HA access to sialic acids expressed by entry receptors and efficient endocytosis. After endosome escape of the viral genome and its migration to the nucleus, replication of the genome, synthesis of viral mRNAs, and translation of viral proteins take place.
New virions assemble at the cell surface and are released from the cell by budding. As the viral components bud from the cell, NA cleaves sialic acids from receptors near the budding site to prevent virions binding back to the dying cell. NA cleavage of sialic acids from the carbohydrate side chains of nascent HA and NA also prevents newly budded virus from clumping together. Both these functions enable efficient release of the nascent virions from the cell.

The relative activity of the HA and NA needs to be balanced to maintain the ability of the virion to efficiently infect and be released from cells. (HA:NA=6:1)



If the HA and NA are mismatched and have mutations in important binding or catalytic sites that alter function, the relative activity of the two proteins may be imbalanced. If the sialidase function of NA is suboptimal, virus may remain bound by decoy receptors, which may shed and block virus entry into the cell. As the virus buds from the cell, an imbalance of HA and NA function may result in the lack of release of the virions due to the binding of HA to the sialic acids expressed at the cell surface that have not been removed by the NA.

The relative activity of the HA and NA needs to be balanced to maintain the ability of the virion to efficiently infect and be released from cells. (HA:NA=6:1)



Alternatively, if the sialidase activity of NA is too strong when compared to the HA-binding activity, sialic acids may be removed from receptors at the expense of the HA being able to bind and trigger endocytosis.

Life Cycle

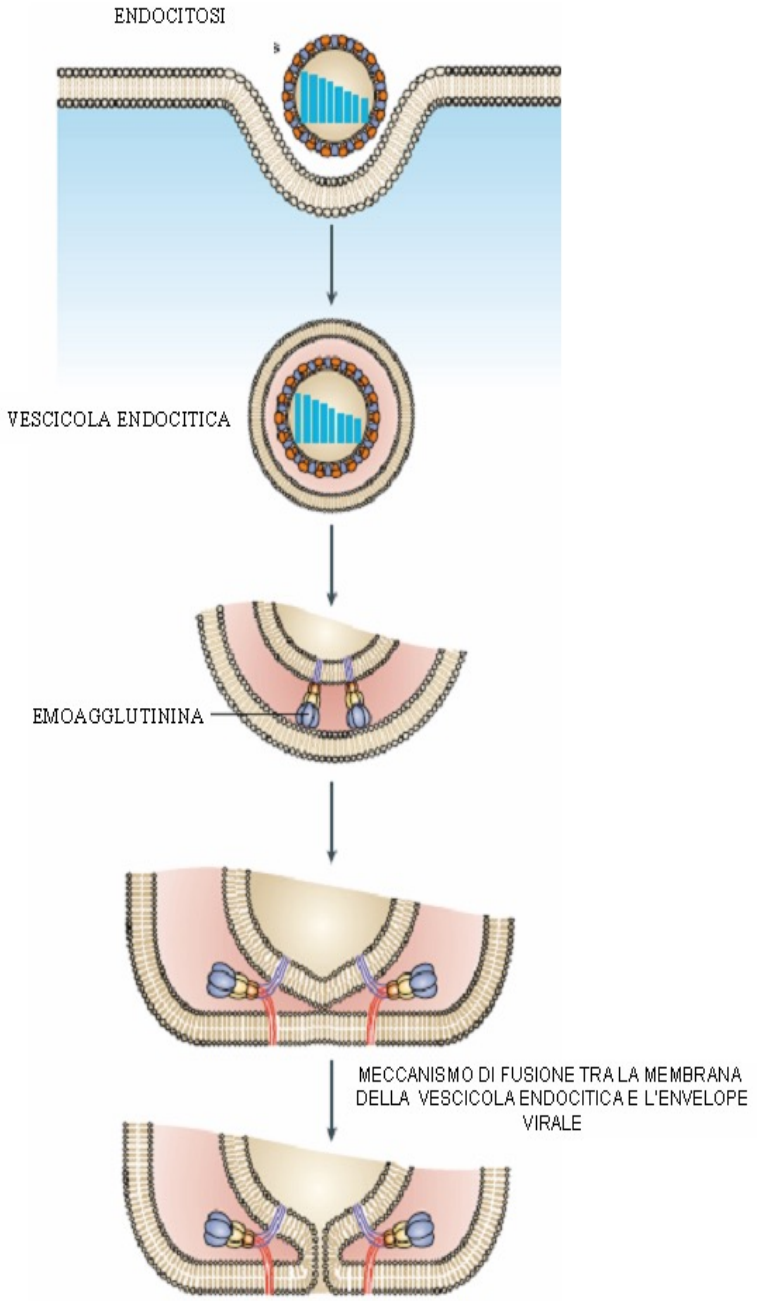
•After binding, the virus is incorporated into an endocytic vesicle. The endosome is acidified by the cell;

•The acidic pH determines a conformational modification of the HA molecules with activation of the fusion domain (located in the HA2 fragment) and subsequent fusion of the viral envelope with the endosome membrane, which determines the passage of the nucleocapsid into the cytoplasm.

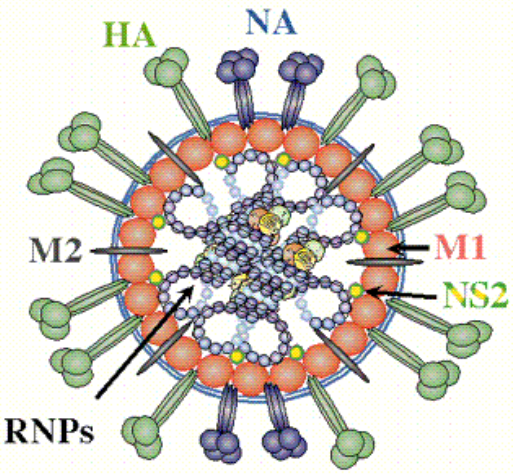
• The ion channels formed by the M2 protein contribute to the virus uncoating.

Life Cycle (endocytosis)

Hemagglutinin mediates the binding of the virus to sialic acid present on the glycoprotein on the cell surface. Through endocytosis, the virus enters the host cell. The acidification of the endocytic vesicle induces a conformational change of the HA exposing the fusion peptide in the HA2 fragment, which thus mediates the fusion between the viral envelope and the membrane of the endocytic vesicle.

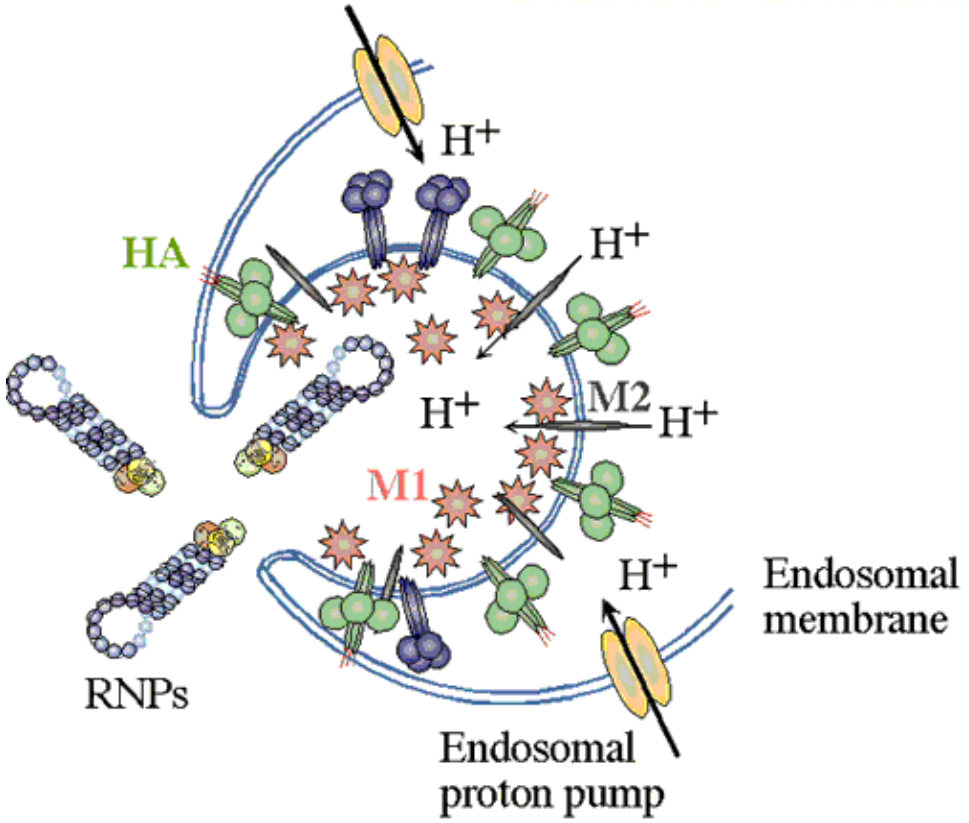


Life cycle (uncoating)

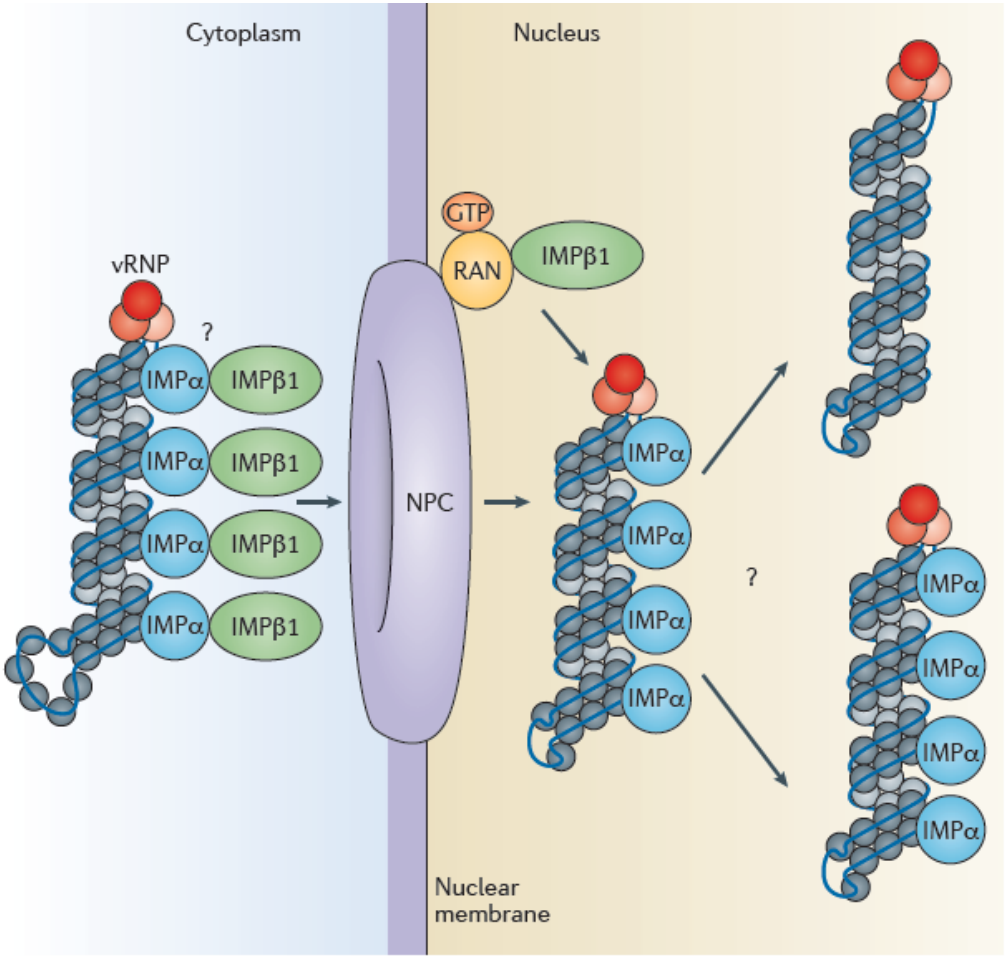


Uncoating and release of vRNP into the cytoplasm

Endocytic vesicle acidification allows proton entry inside the virion through the M2 ion channels. Virion acidification induces detachment of vRNP from the M1 matrix protein and their release into the cytoplasm.



Model for vRNP nuclear import

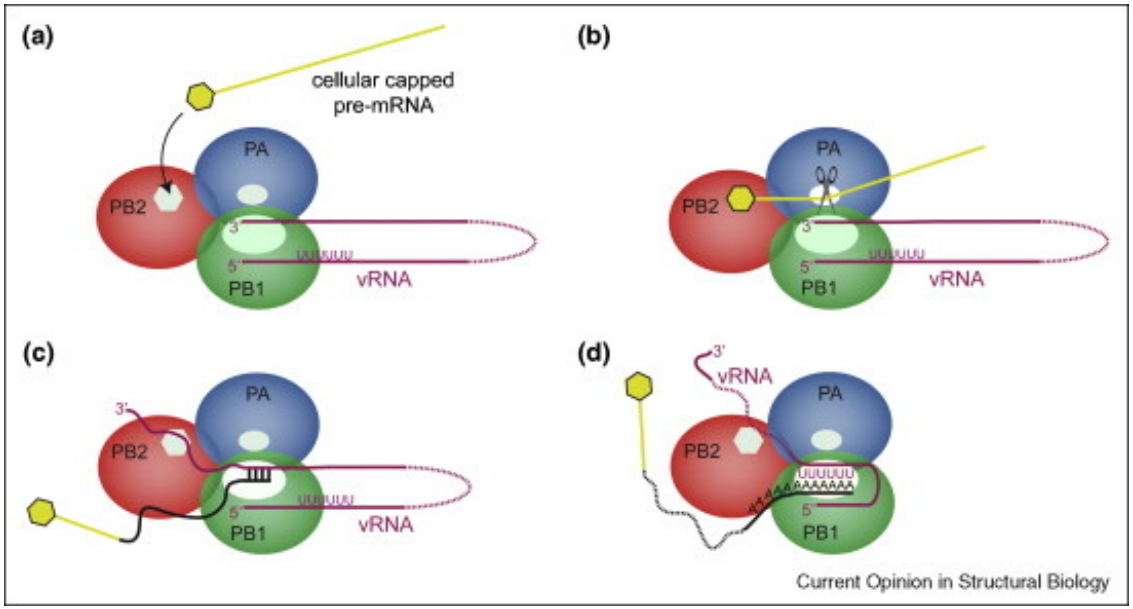


Uncoated, cytoplasmic viral ribonucleoproteins (vRNPs) with exposed nucleoprotein (NP) nuclear localization sequence (NLS) motifs associate with importin-α (IMPα), which in turn associates with IMPβ1. The entire complex docks at the nuclear pore complex (NPC) and is transported into the nucleus, where RAN–GTP binds to IMPβ1 and facilitates vRNP release into the nucleoplasm to initiate transcription and replication. Whether multiple IMPα and IMPβ1 molecules associate with each vRNP is unknown, as is the fate of the vRNP-associated IMPα once the vRNP cargo is released into the nucleoplasm.

Nature Reviews Microbiology **volume 13**, pages 28–41 (2015)

LIFE CYCLE (Transcription)

- Genome segments are transcribed by the polimerase complex (composed by PA, PB1 e PB2) associated to each genome segment. PB2: cap binding, PB1: initiation and elongation, PA: endonuclease activity

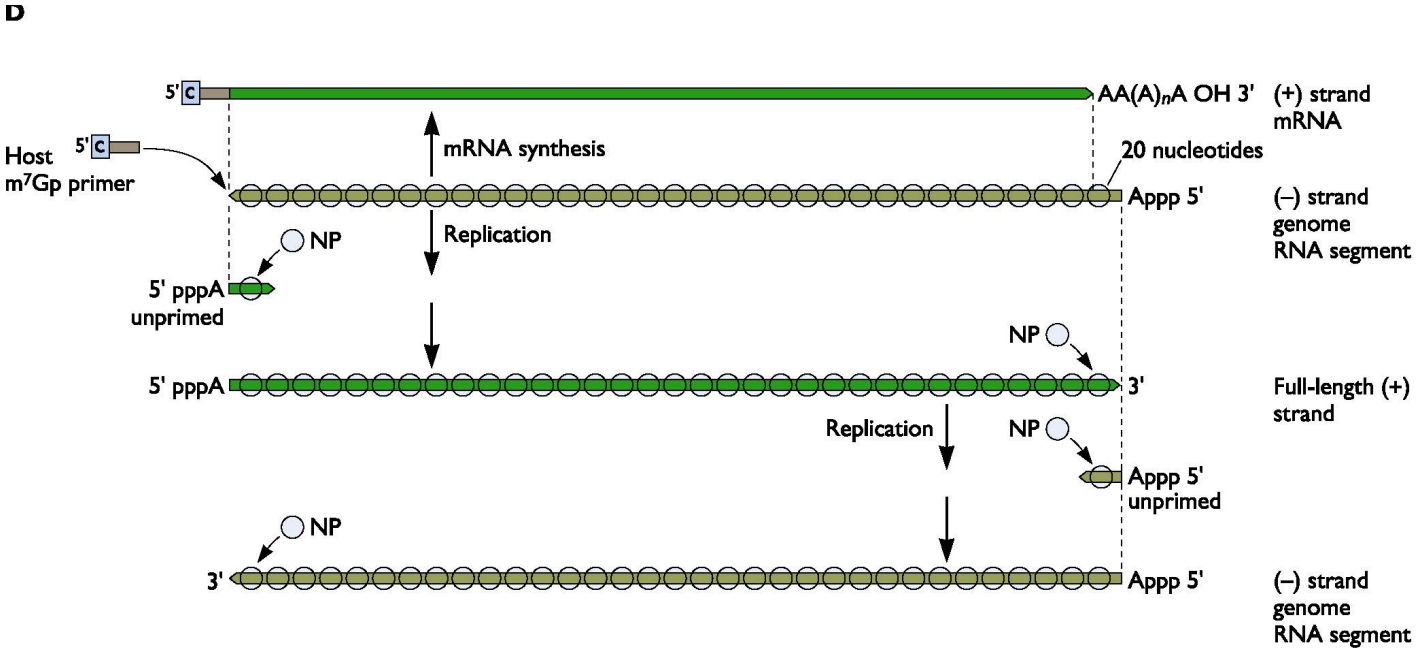


Schematic diagram showing steps in cap-dependent transcription by influenza virus polymerase. (a) Binding of host pre-mRNAs (yellow) by the cap binding domain located in the PB2 subunit. (b) Cleavage of the host mRNA after 10–13 nucleotides by the endonuclease located in the PA subunit. (c) Elongation of the chimeric viral mRNA by the nucleotidyl-transferase site in the PB1 subunit using the vRNA as template. (d) Poly-adenylation of the viral mRNA by polymerase stuttering at the oligo-U sequence near the 5' end of the vRNA.

Current Opinion in Structural Biology 2010, 20:104–113

LIFE CYCLE (genome replication)

•The transition from transcription to replication of genomic RNA depends, in part, on the abundance of the NP protein and on the polymerase acquisition of the ability to initiate a primer-independent RNA synthesis.



From Flint et al. *Principles of Virology* (2000), ASM Press

Article

Host ANP32A mediates the assembly of the influenza virus replicase

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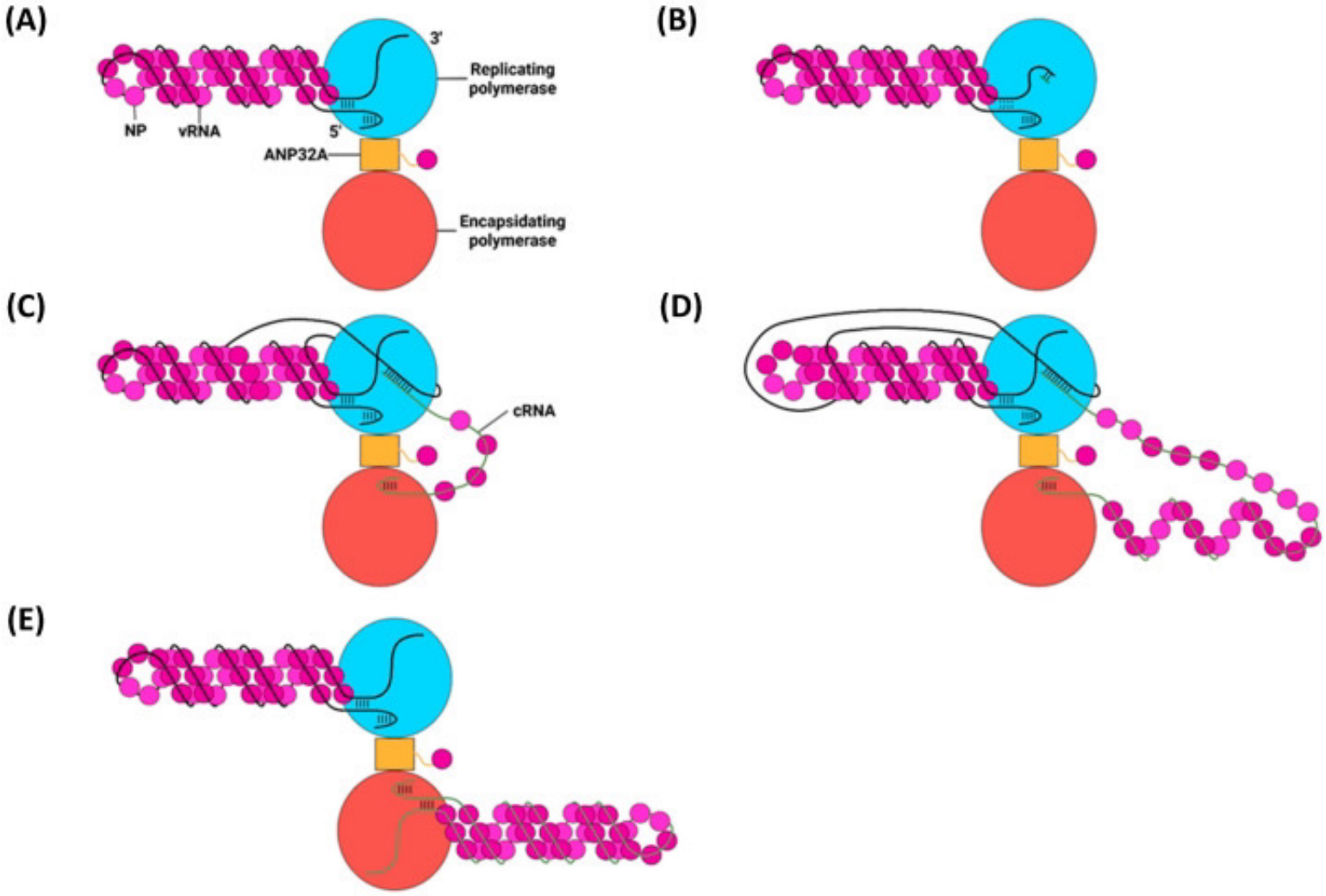
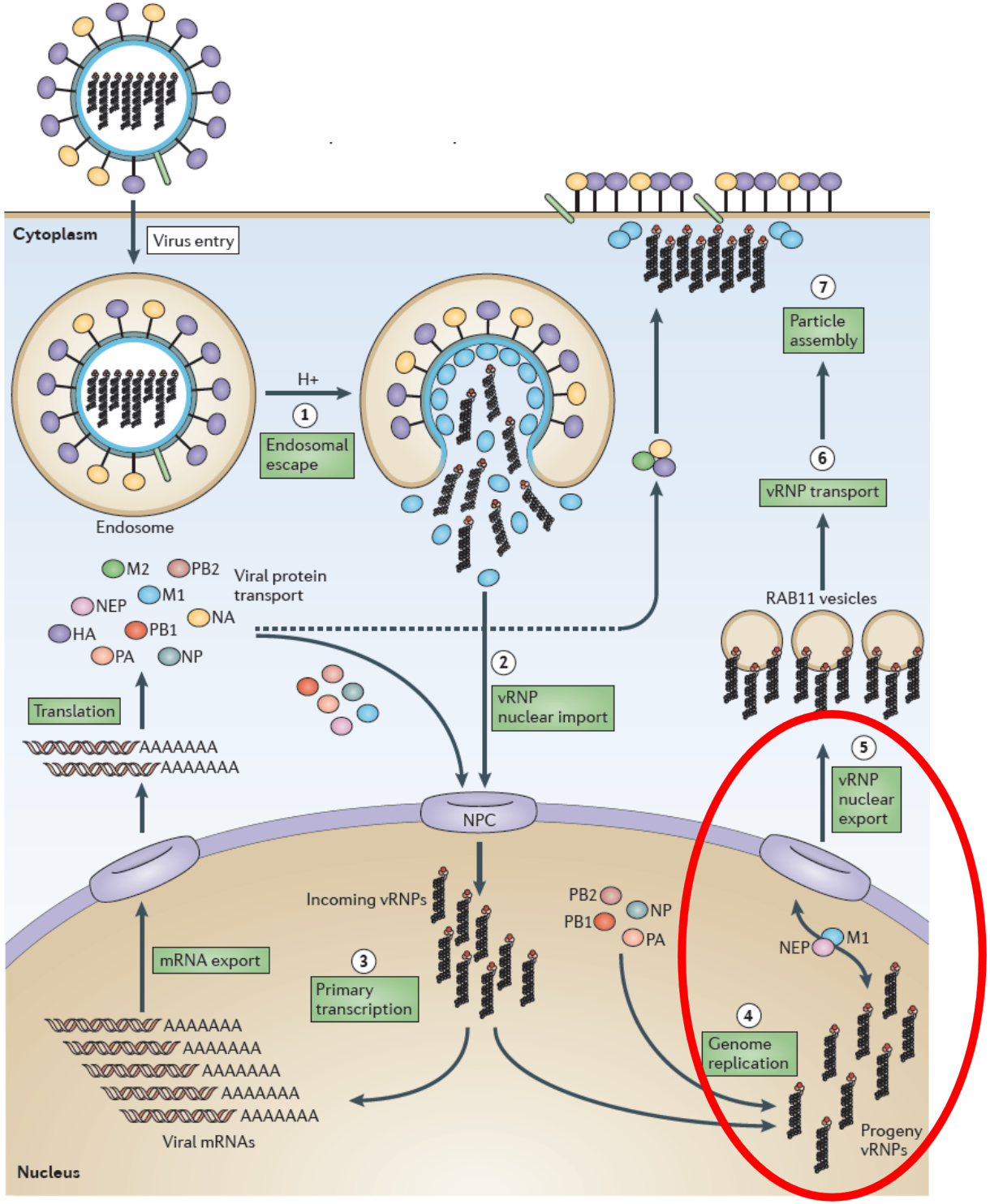
Aquatic birds represent a vast reservoir from which new pandemic influenza A viruses can emerge[1]. Influenza viruses contain a negative-sense segmented RNA genome that is transcribed and replicated by the viral heterotrimeric RNA polymerase (FluPol) in the context of viral ribonucleoprotein complexes[2,3]. RNA polymerases of avian influenza A viruses (FluPolA) replicate viral RNA inefficiently in human cells because of species-specific differences in acidic nuclear phosphoprotein 32 (ANP32), a family of essential host proteins for FluPol activity[4]. Host-adaptive mutations, particularly a glutamic-acid-to-lysine mutation at amino acid residue 627 (E627K) in the 627 domain of the PB2 subunit, enable avian FluPolA to overcome this restriction and efficiently replicate viral RNA in the presence of human ANP32 proteins. However, the molecular mechanisms of genome replication and the interplay with ANP32 proteins remain largely unknown. Here we report cryo-electron microscopy structures of influenza C virus polymerase (FluPolC) in complex with human and chicken ANP32A. In both structures, two FluPolC molecules form an asymmetric dimer bridged by the N-terminal leucine-rich repeat domain of ANP32A. The C-terminal low-complexity acidic region of ANP32A inserts between the two juxtaposed PB2 627 domains of the asymmetric FluPolA dimer, suggesting a mechanism for how the adaptive PB2(E627K) mutation enables the replication of viral RNA in mammalian hosts. We propose that this complex represents a replication platform for the viral RNA genome, in which one of the FluPol molecules acts as a replicase while the other initiates the assembly of the nascent replication product into a viral ribonucleoprotein complex.

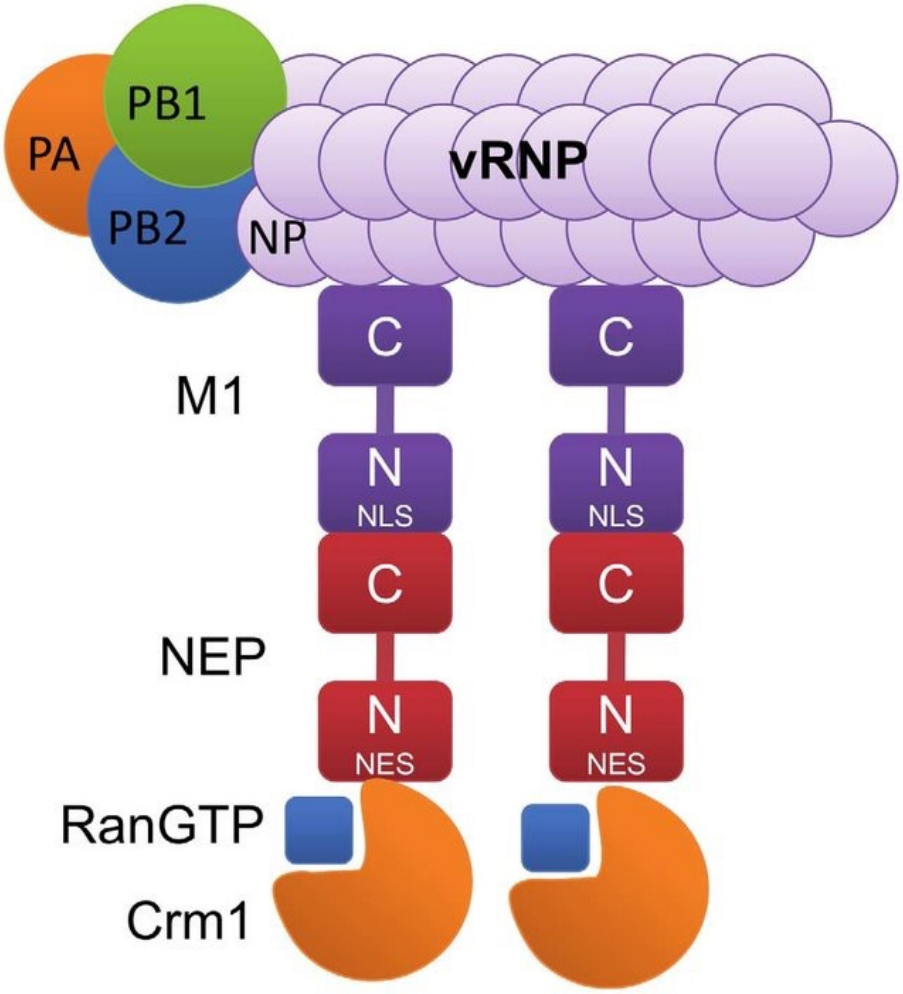
Primary replication at the level of vRNPs.



Life Cycle

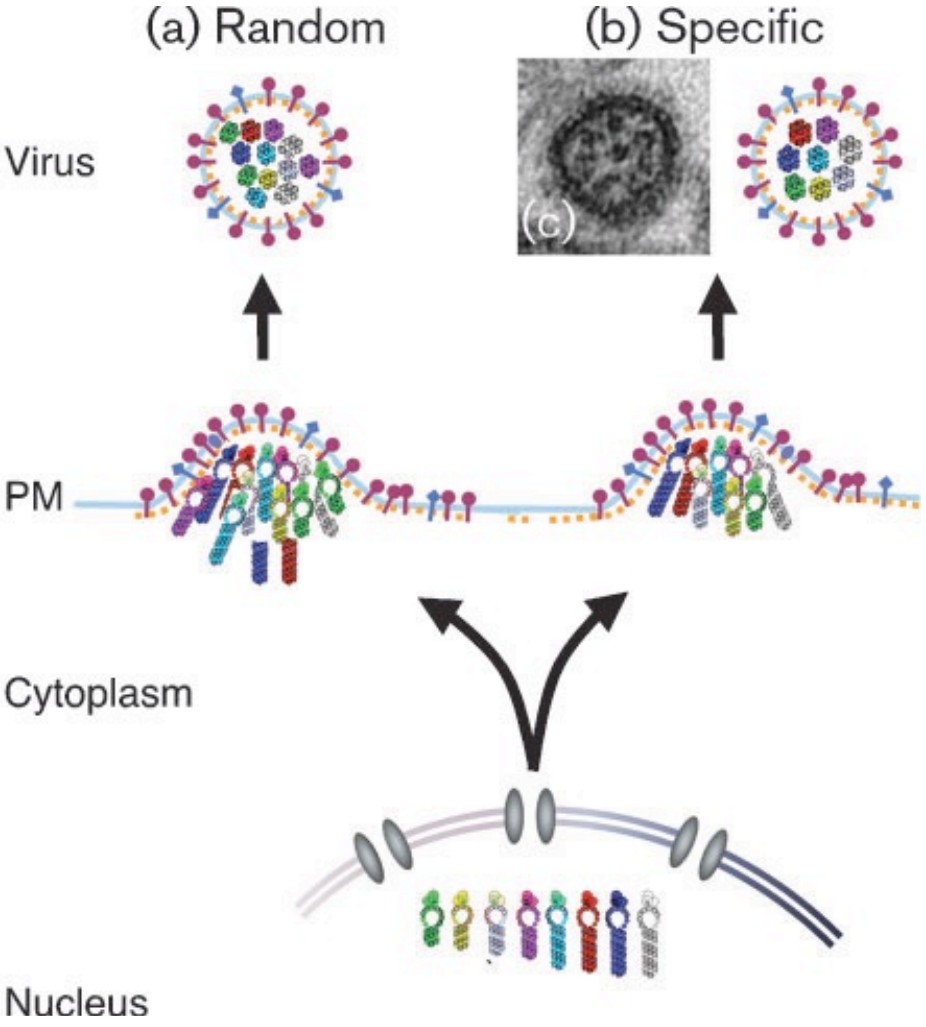

Cytoplasm
Virus entry
Endosome
H+
1
Endosomal escape
7
Particle assembly
6
vRNP transport
RAB11 vesicles
M2
PB2
M1
NEP
NA
HA
PB1
PA
NP
Viral protein transport
Translation
AAAAAAA
AAAAAAA
2
vRNP nuclear import
5
vRNP nuclear export
NPC
Incoming vRNPs
PB2
PB1
NP
PA
NEP
M1
mRNA export
3
Primary transcription
4
Genome replication
AAAAAAA
AAAAAAA
AAAAAAA
AAAAAAA
AAAAAAA
Viral mRNAs
Progeny vRNPs
Nucleus

vRNP nuclear export



NEP-mediated nuclear export of influenza virus vRNPs. The β-importin Crm1 mediates export of the vRNP complex by binding to the N-terminal domain of NEP, as well as to its cofactor, the small GTPase Ran. The C-terminus of NEP binds to the nuclear localisation signal (NLS) on the N-terminal domain of the viral matrix protein M1. The C-terminus of M1 in turn binds strongly to the vRNP through interaction with NP

Genome packaging



The eight individual segments (differentiated by colour) are replicated independently in the nucleus before being exported to the cytoplasm and migrating to the apical plasma membrane (PM). There, they interact with other viral structural proteins and new virus particles form by budding. (a) The random model for genome packaging proposes that more than eight RNPs are incorporated in a segment non-specific manner such that a reasonable proportion of virions contain at least one copy of each segment. (b) The specific model proposes that unique segment specific packaging signals operate to form a defined array of eight RNPs containing one copy of each segment. (c) A negatively stained EM section through an influenza virion showing the distinctive 7+1 array of RNPs.

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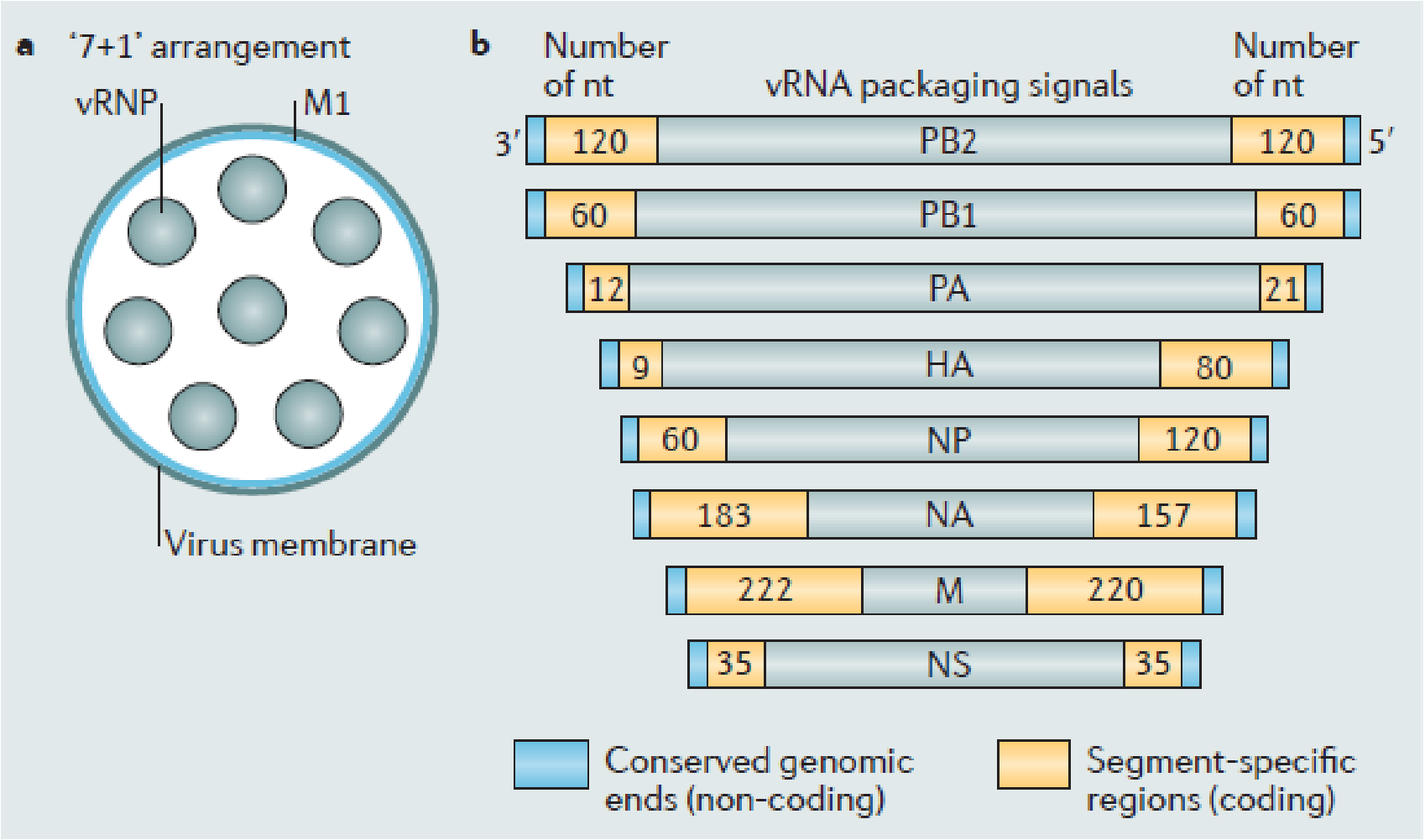
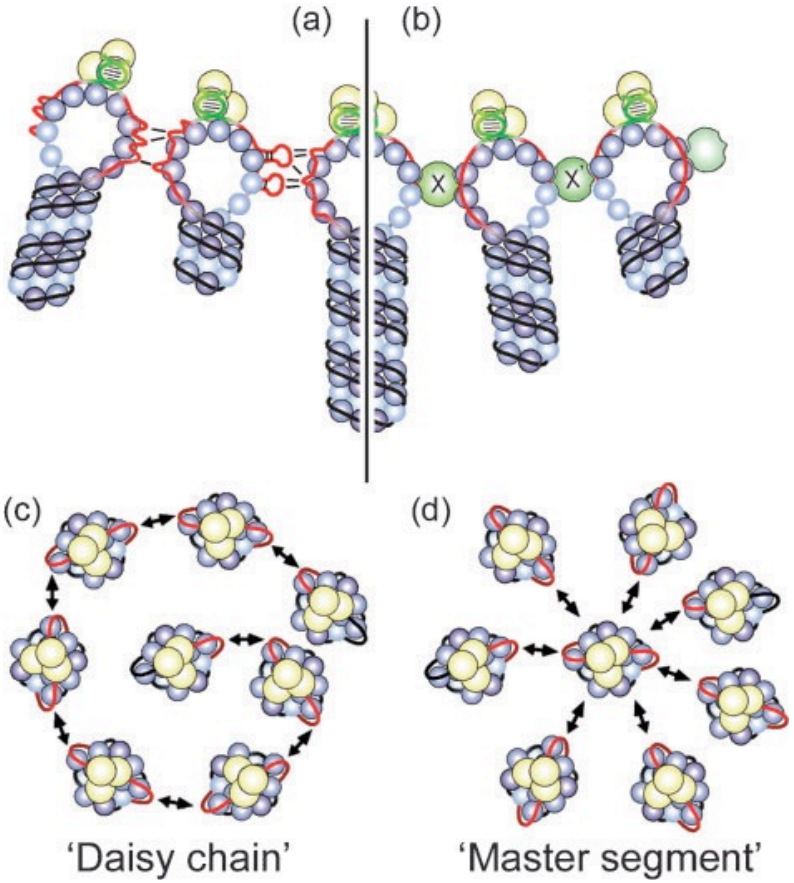
The packaging signals



Models of possible selective packaging methods for influenza A virus

It is proposed that the selective packaging of the segments is brought about by the assembly of a non-covalently linked higher-order genome complex, containing each of the eight vRNAs. The formation of this complex is mediated by specific interactions between the packaging signals of the segments, either (a) by direct RNA–RNA interactions between the packaging signals, possibly with the involvement of short secondary structures or (b) by as-yet-unidentified protein factors (X, X' etc).
(c, d) Possible organizations of the 7+1 genome complex utilizing the minimum possible number of between-segment interactions (arrows).



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ARTICLE

OPEN

Selective flexible packaging pathways of the segmented genome of influenza A virus

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The genome of influenza A viruses (IAV) is encoded in eight distinct viral ribonucleoproteins (vRNPs) that consist of negative sense viral RNA (vRNA) covered by the IAV nucleoprotein. Previous studies strongly support a selective packaging model by which vRNP segments are bundling to an octameric complex, which is integrated into budding virions. However, the pathway(s) generating a complete genome bundle is not known. We here use a multiplexed FISH assay to monitor all eight vRNAs in parallel in human lung epithelial cells. Analysis of 3.9 × 105 spots of colocalizing vRNAs provides quantitative insights into segment composition of vRNP complexes and, thus, implications for bundling routes. The complexes rarely contain multiple copies of a specific segment. The data suggest a selective packaging mechanism with limited flexibility by which vRNPs assemble into a complete IAV genome. We surmise that this flexibility forms an essential basis for the development of reassortant viruses with pandemic potential.

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Packaging signal of influenza A virus

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Influenza A virus (IAV) contains a genome with eight single-stranded, negative-sense RNA segments that encode 17 proteins. During its assembly, all eight separate viral RNA (vRNA) segments are incorporated into virions in a selective manner. Evidence suggested that the highly selective genome packaging mechanism relies on RNA-RNA or protein- RNA interactions. The specific structures of each vRNA that contribute to mediating the packaging of the vRNA into virions have been described and identified as packaging signals. Abundant research indicated that sequences required for genome incorporation are not series and are varied among virus genotypes. The packaging signals play important roles in determining the virus replication, genome incorporation and genetic reassortment of influenza A virus. In this review, we discuss recent studies on influenza A virus packaging signals to provide an overview of their characteristics and functions.

Keywords: Influenza A virus, Packaging signals, Incorporation, Reassortment, RNA-RNA interaction