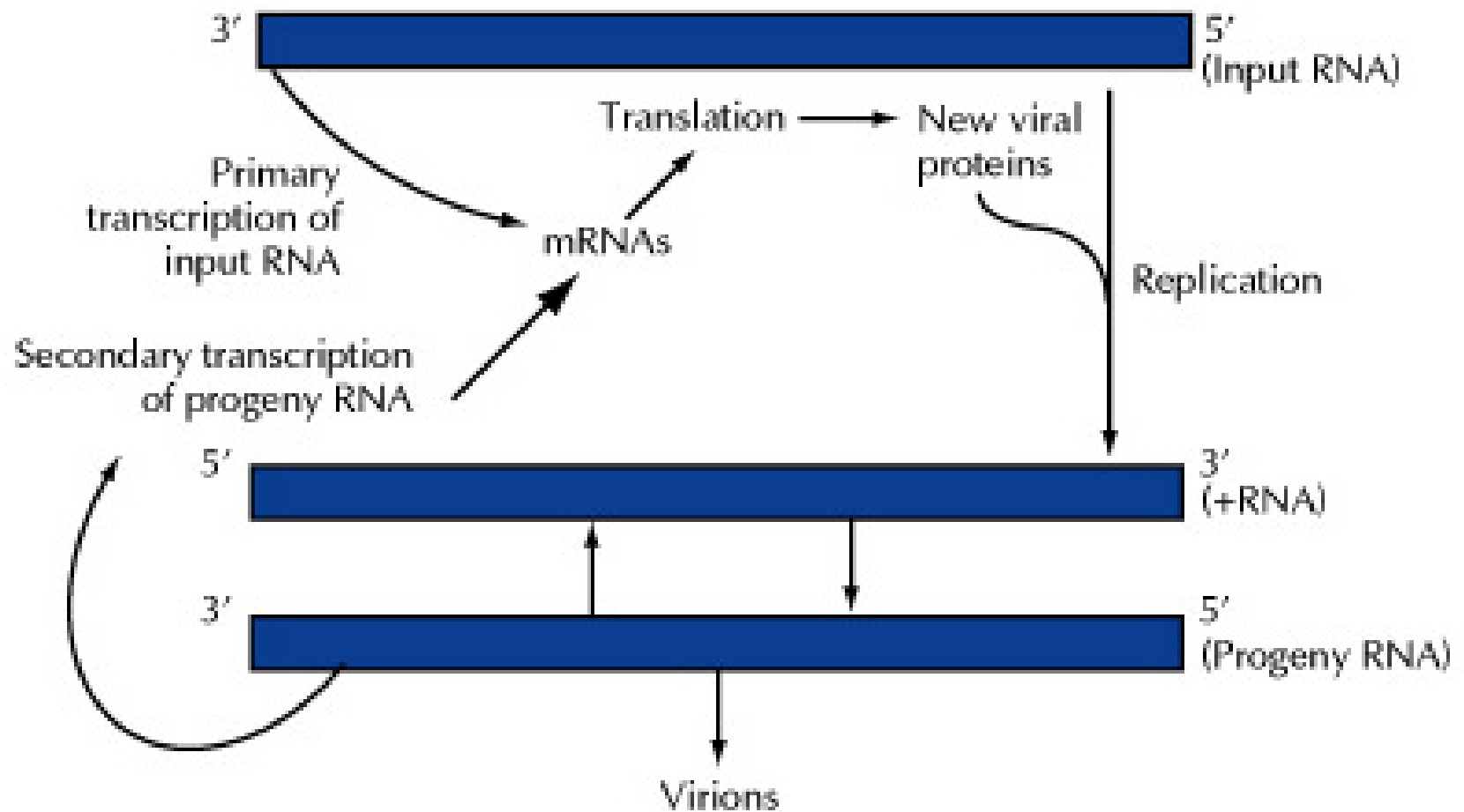


VIROLOGIA 2006/2007

APRESENTAÇÃO 7 **(Vírus de RNA-)**

Maria Filomena Caeiro



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RHABDOVIRIDAE

Rhabdoviruses

Rabies virus

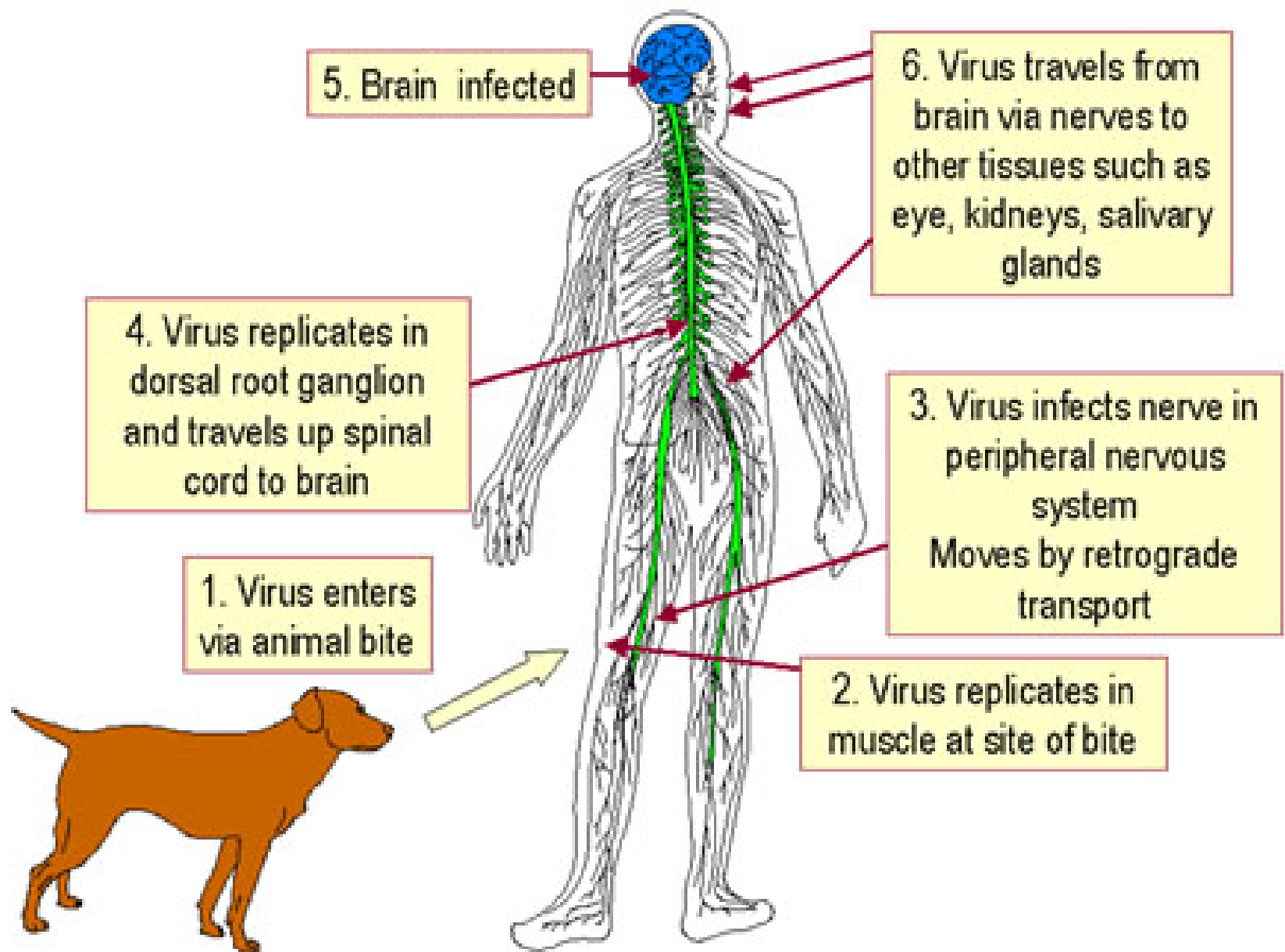
Virus	Disease	Epidemiology	
Lyssavirus • Rabies virus • Related viruses of rodents and bats	Rabies Rarely cause rabies-like encephalitis	Transmission • Reservoir: wild animals • Vectors: wild animals, unvaccinated dogs, cats • Bite of rabid animal (virus in saliva) or aerosols in caves harboring rabid bats	
Vesiculovirus • Vesicular stomatitis virus	Flu-like illness	At risk or risk factors • Animal handlers, veterinarians • Those in countries with no pet vaccinations or quarantine	
		Distribution of virus • Ubiquitous, except certain islands • No seasonal incidence	
		Vaccines or antiviral drugs • Vaccines for pets and wild animals • Inactivated virus vaccine for at-risk personnel, postexposure prophylaxis • No antiviral drugs	

•*Rhabdovirus* de plantas:

•Potato yellow dwarf

•Lettuce necrotic yellows

Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.



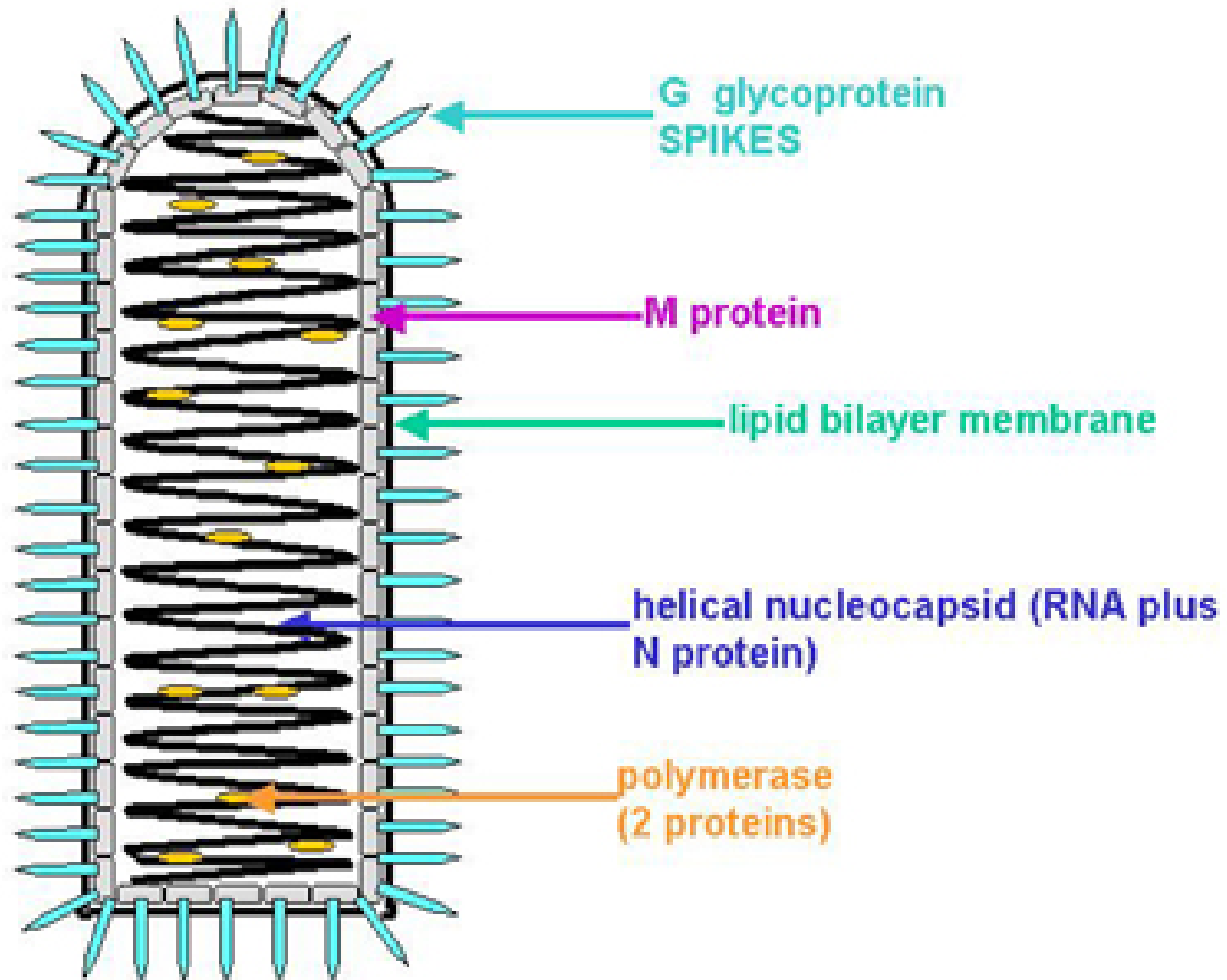
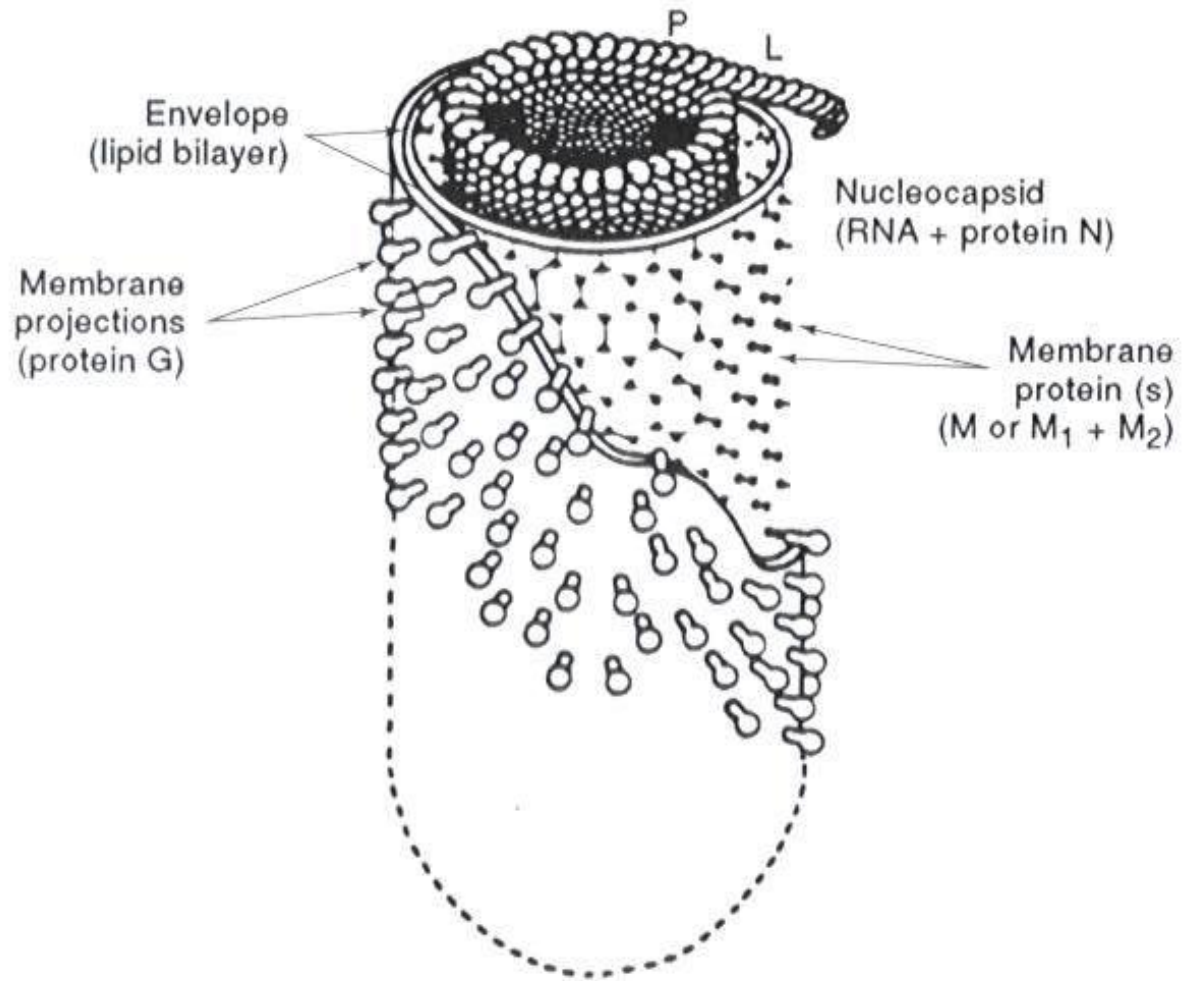


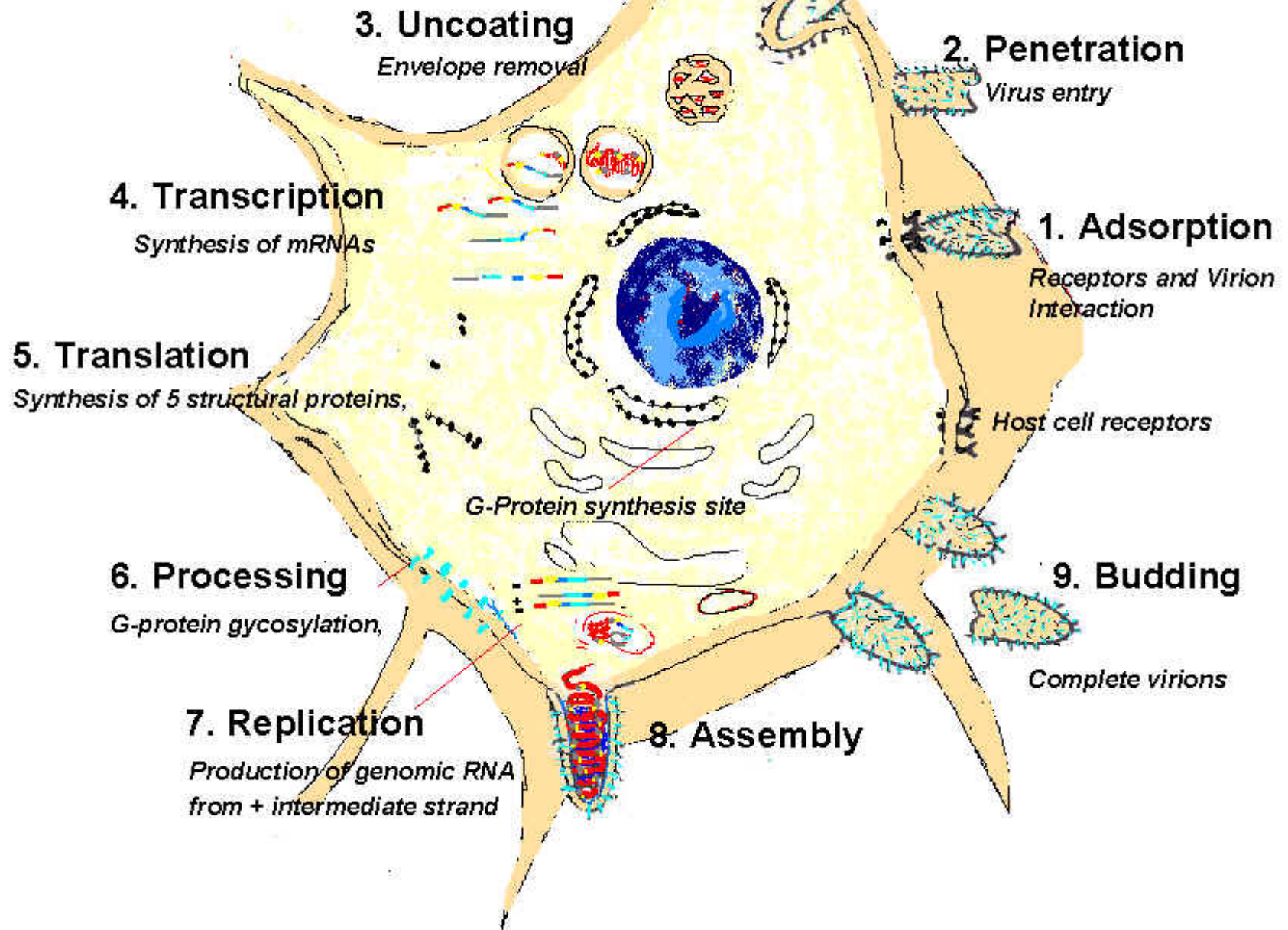
FIGURE 4.2

Model of a rhabdovirus particle cut through the middle to expose the internal structures. Bar = 10 nm. (After R. I. B. Francki and J. W. Randles. In *Rhabdoviruses*, ed. D. H. L. Bishop (1980) Vol. 3, 135 CRC Press, Boca Raton, Fla.)

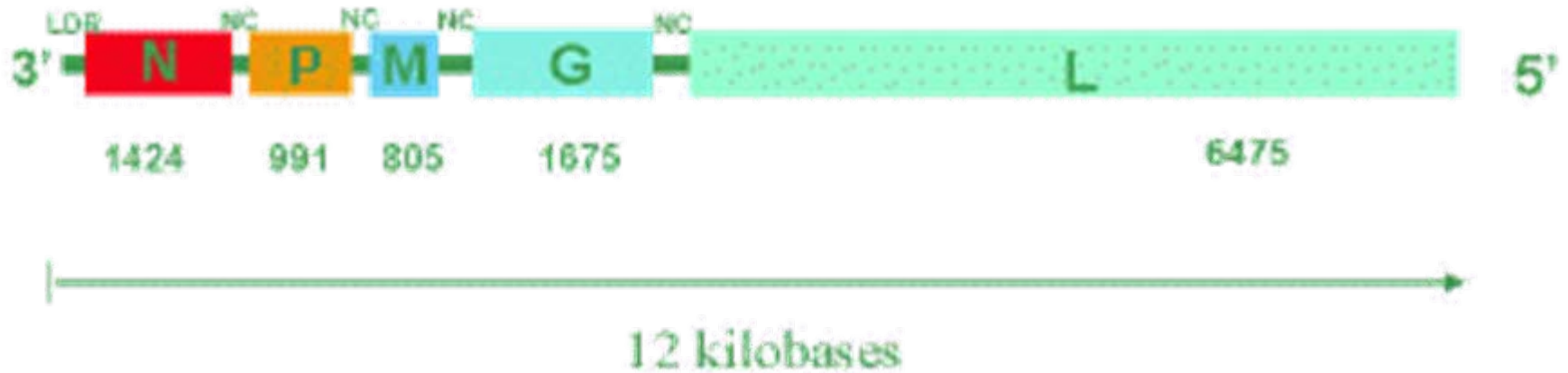


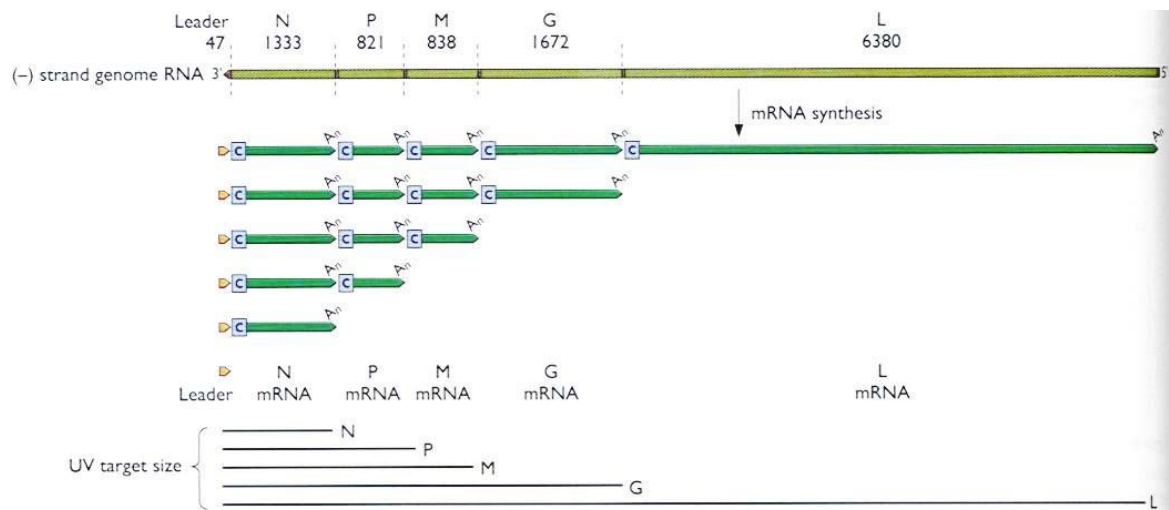
Levy, J. A., Fraenkel-Conrat, H. and Owens, R. A. (1994). "Virology". 3rd edition. Prentice-Hall, Inc.

Cycle of Infection and Replication



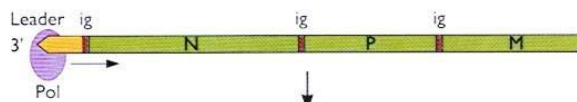
Rabies Genome



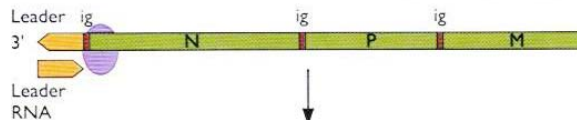


B

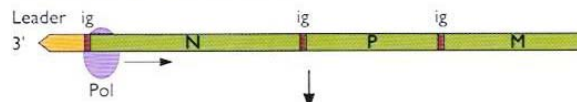
Initiation at 3' end of VSV genome RNA



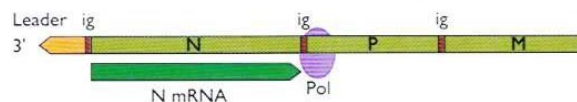
Synthesize leader and terminate at intergenic region (ig)



Reinitiate at 3' end of N gene



Synthesize N gene and terminate at intergenic region (ig)



Reinitiate at 3' end of P gene

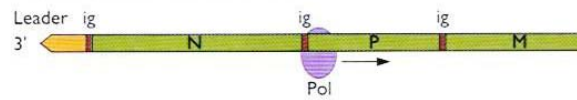


Figure 6.11 Vesicular stomatitis virus RNA synthesis. (A) Vesicular stomatitis virus mRNA map and UV map. The genome is shown as a thick green line at the top, and the N, P, M, G, and L genes and their relative sizes are indicated. The 47-nucleotide leader RNA is encoded at the 3' end of the genomic RNA. The leader and intergenic regions are shown in red. The RNAs encoded at the 3' end of the genome are made in larger quantities than the RNAs encoded at the 5' end of the genome. UV irradiation experiments determined the size of the vesicular stomatitis virus genome (UV target size) required for synthesis of each of the viral mRNAs. The UV target size of each viral mRNA corresponded to the size of the genomic RNA sequence encoding the mRNA plus all of the genomic RNA sequence 3' to this coding sequence. (B) Stop-start model of mRNA synthesis. The RNA polymerase (Pol) initiates RNA synthesis at the 3' end of the genome RNA. The leader RNA is elongated, and synthesis terminates at the intergenic region (ig). RNA synthesis reinitiates at the 3' end of the N gene, and after synthesis of the N mRNA, RNA synthesis terminates at the intergenic region, followed by reinitiation at the 3' end of the P gene. This process continues until all five mRNAs are synthesized. Reinitiation does not occur after the last mRNA (the L mRNA) is synthesized, and as a consequence the 59 5'-terminal nucleotides of the vesicular stomatitis virus genomic RNA are not copied. Only a fraction of the polymerase molecules successfully make the transition from termination to reinitiation of mRNA synthesis at each intergenic region. (C) Functions of the RNA polymerase at an intergenic region. After the U7 sequence at the end of the mRNA-encoding sequence has been copied, the resulting A7 sequence in the mRNA slips off the U7 genomic sequence, which is then recopied. This process continues until approximately 200 A residues are added to the 3' end of the mRNA. Termination then occurs, completing the synthesis of the mRNA, followed by initiation and capping of the next mRNA. The dinucleotide NA in the genomic RNA is not copied. The

Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

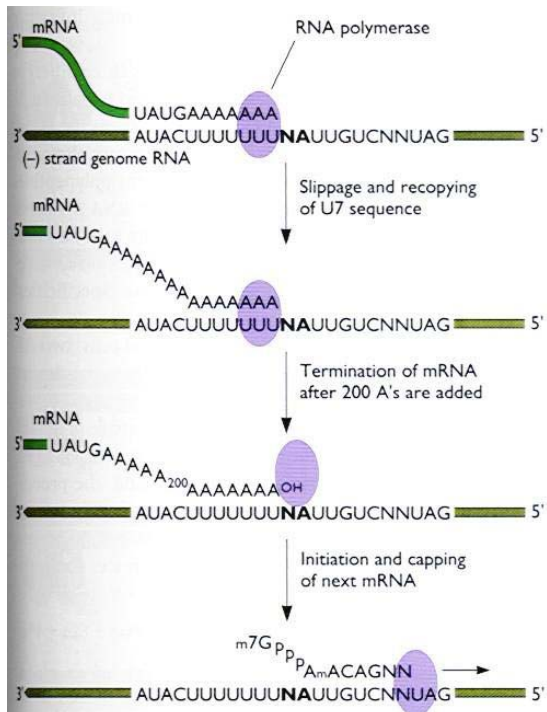
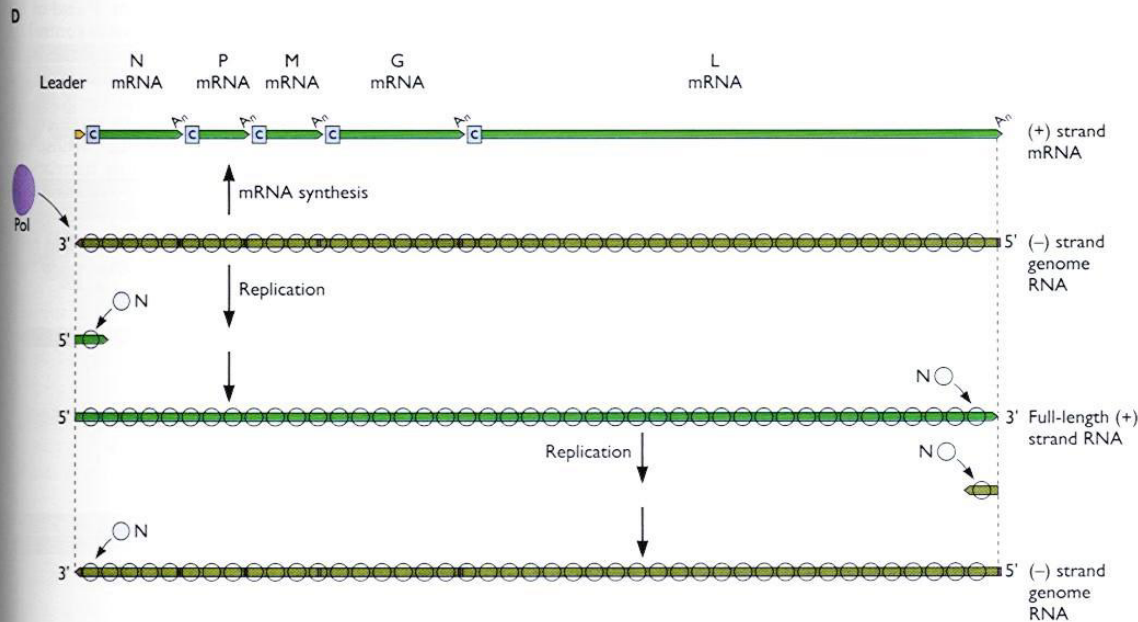


Figure 6.11 (continued)

genomic RNA being copied is bound to viral N protein molecules at regular intervals (15 to 20 nucleotides). (D) The switch from mRNA synthesis to genomic RNA replication is regulated by the viral nucleocapsid (N) protein. When concentrations of N protein are low, mRNA synthesis is favored (top). When the N protein concentration is higher, it binds to nascent (+) strands and allows the RNA polymerase to read through the intergenic junctions (ig) at which polyadenylation and termination take place during mRNA synthesis.



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

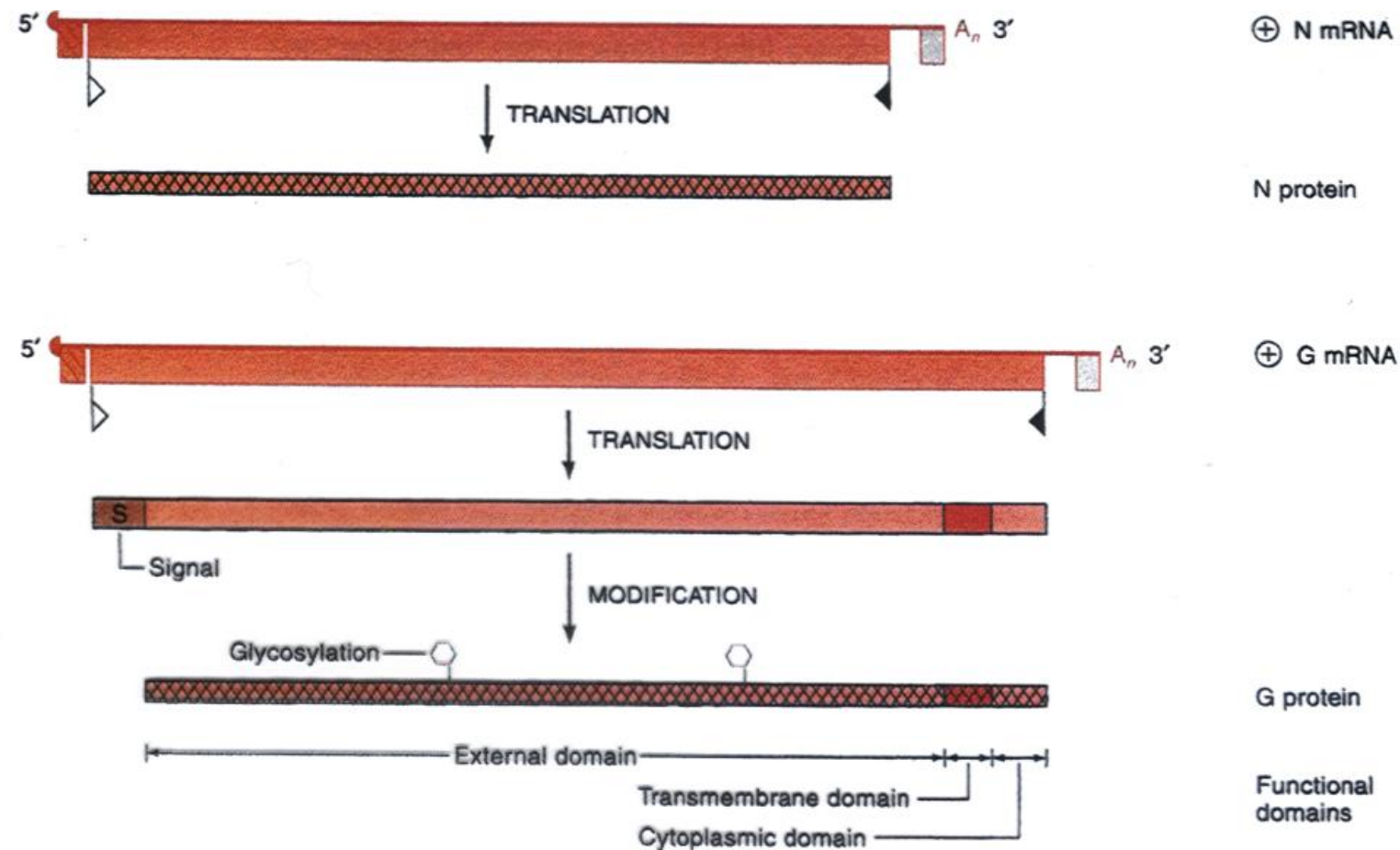


Figure 2.20

Generic translation strategy of monocistronic mRNAs. Two mRNAs from VSV and their translation products are shown. N is the nucleocapsid protein and G is the glycoprotein. The diagonally hatched box at the 5' end is a short conserved nucleotide motif; the shaded box at the 3' end is a polyadenylation signal. Functional domains of the protein are labelled; the open hexagons represent carbohydrate chains which are added post-translationally.

Ordem: *Mononegavirales*

Família: *Paramyxoviridae*

Sub-família: *Paramyxovirinae*

Respirovirus

Rubulavirus

Morbillivirus

Sub-família: *Pneumovirinae*

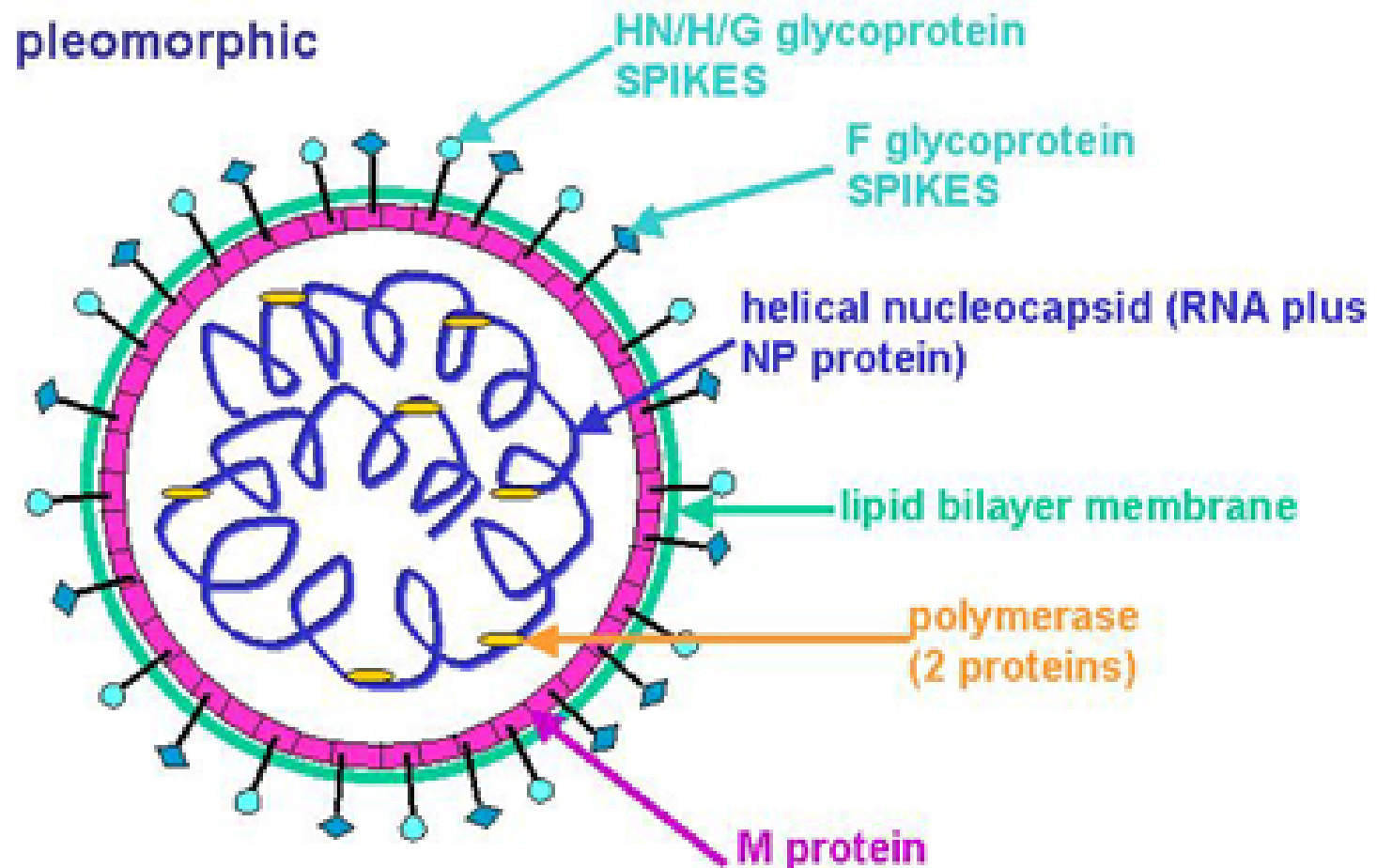
Pneumovirus

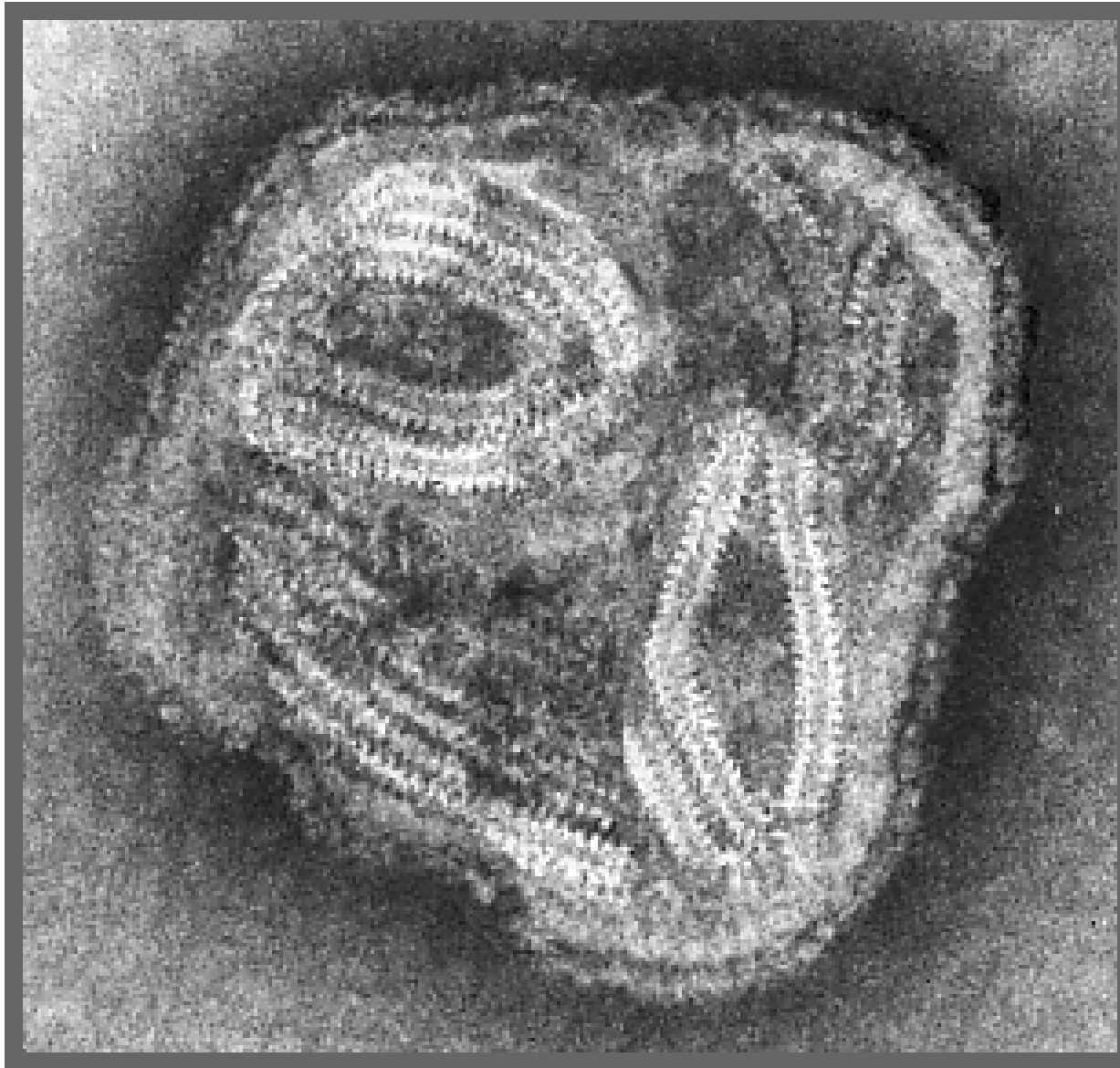
Metapneumovirus

TABLE 1

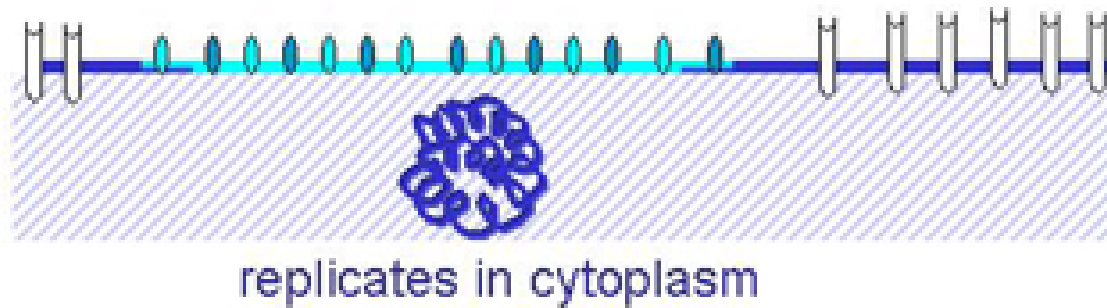
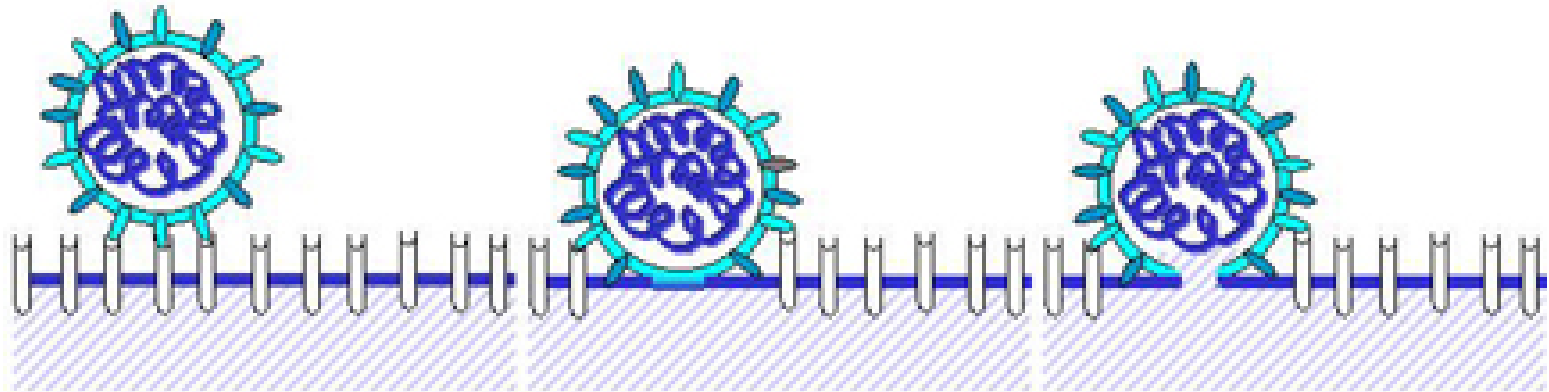
GENUS	MEMBERS	GLYCOPROTEINS
Paramyxovirus	human parainfluenza virus1 (HPIV 1) human parainfluenza virus3 (HPIV 3)	HN, F
Rubulavirus	human parainfluenza virus2 (HPIV 2) human parainfluenza virus4 (HPIV 4) Mumps virus	HN, F
Morbillivirus	measles	H, F
Pneumovirus	respiratory syncytial virus	G, F

PARAMYXOVIRUSES





PENETRATION - FUSION



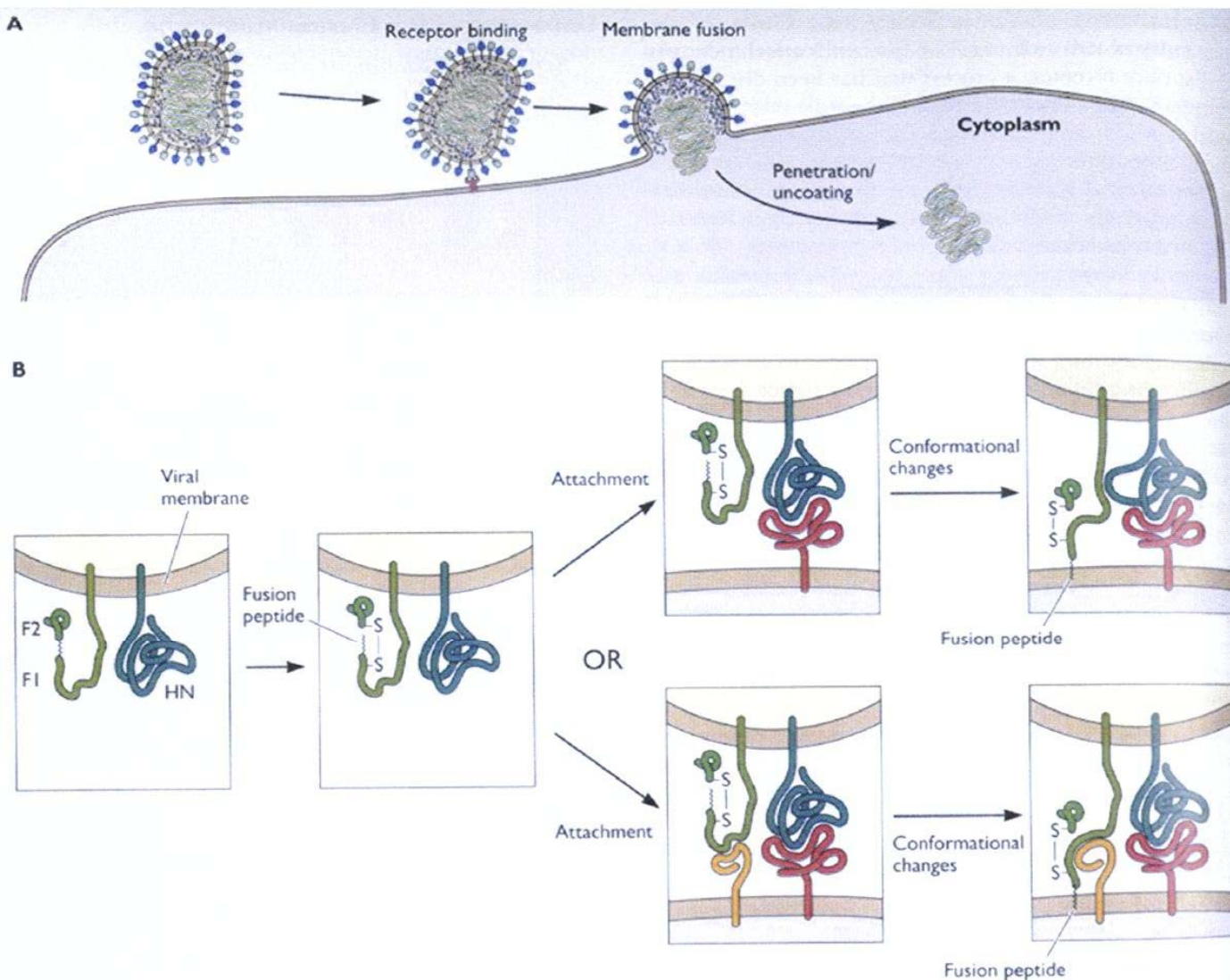


Figure 5.4 Penetration and uncoating at the plasma membrane. (A) Overview. Members of the *Paramyxoviridae* bind to cell surface receptors via HN, H, or G glycoprotein. The fusion protein (F) then catalyzes membrane fusion at the cell surface at neutral pH. The viral nucleocapsid, a ribonucleoprotein complex, is then released into the cytoplasm, where RNA synthesis begins. The mechanism by which contacts between the viral nucleocapsid and the M protein, which forms a shell beneath the lipid bilayer, are broken to facilitate release of the nucleocapsid into the cytoplasm is not known. (B) Models for F-protein-mediated membrane fusion. In one model, binding of HN to the cell receptor (red) induces conformational changes in HN which in turn induce conformational changes in the F protein, moving the fusion peptide from a buried position nearer to the cell membrane. In another model, interaction of HN with a cell receptor leads to the binding of F to a putative receptor (orange), which induces conformational changes in F, exposing the fusion peptide.

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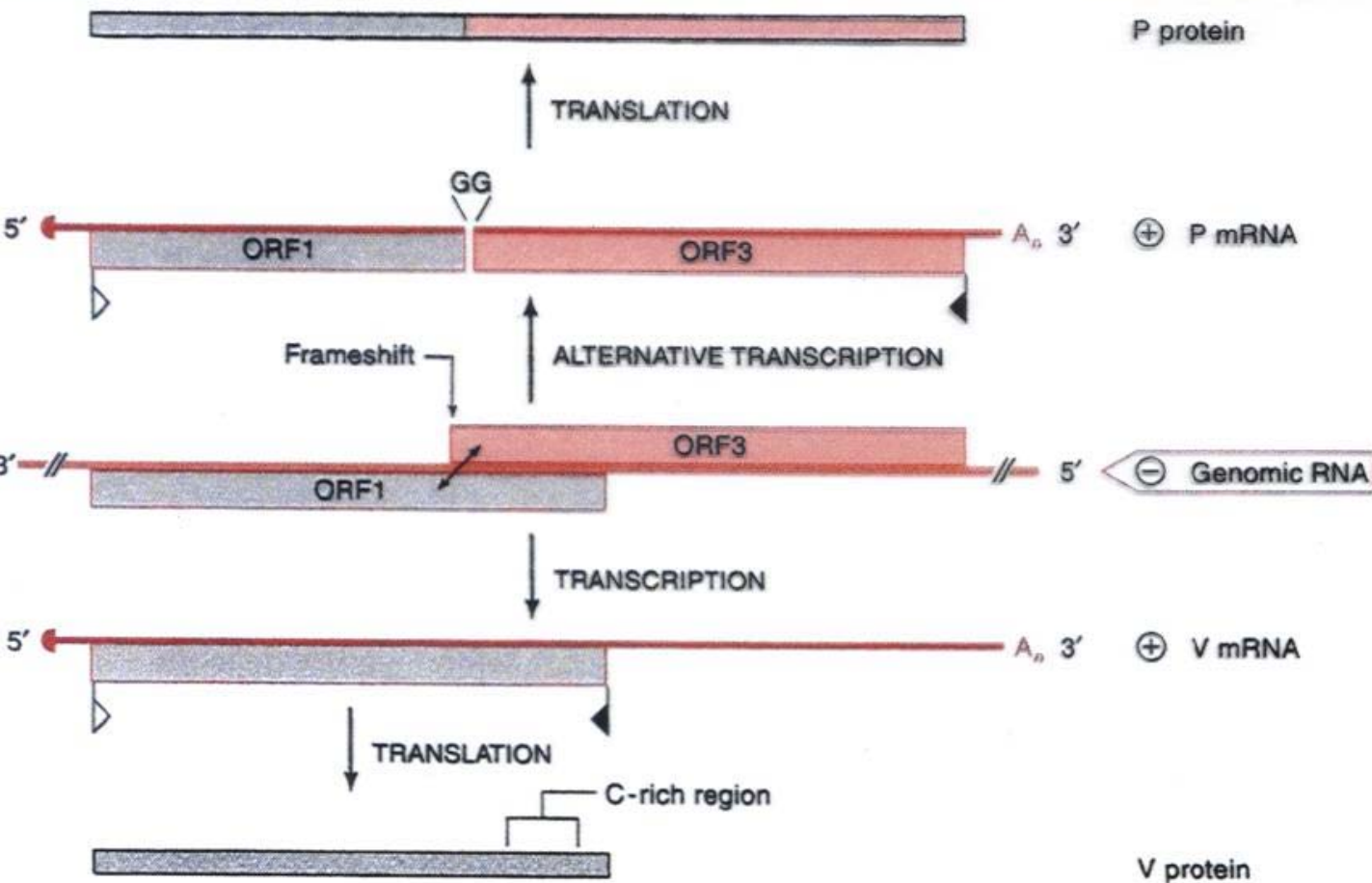


Figure 2.22

Nontemplated nucleotides in paramyxovirus mRNAs. Transcription of the P gene of SV5 to produce the P and V proteins is shown. Transcription of V mRNA begins by internal initiation on the paramyxovirus genome, and translation from the first AUG to the first stop codon (ORF1) to produce the V protein. Alternatively, two nontemplated G residues are added at a defined position during transcription to produce the P mRNA, which is translated into the P protein. Although the amino termini of the two proteins share an amino acid sequence, the characteristics of the proteins downstream of the insertion are



transcription

replication (protein synthesis is a pre-requisite)

5 mRNAs (+ve sense)

full-length copy



* = cap

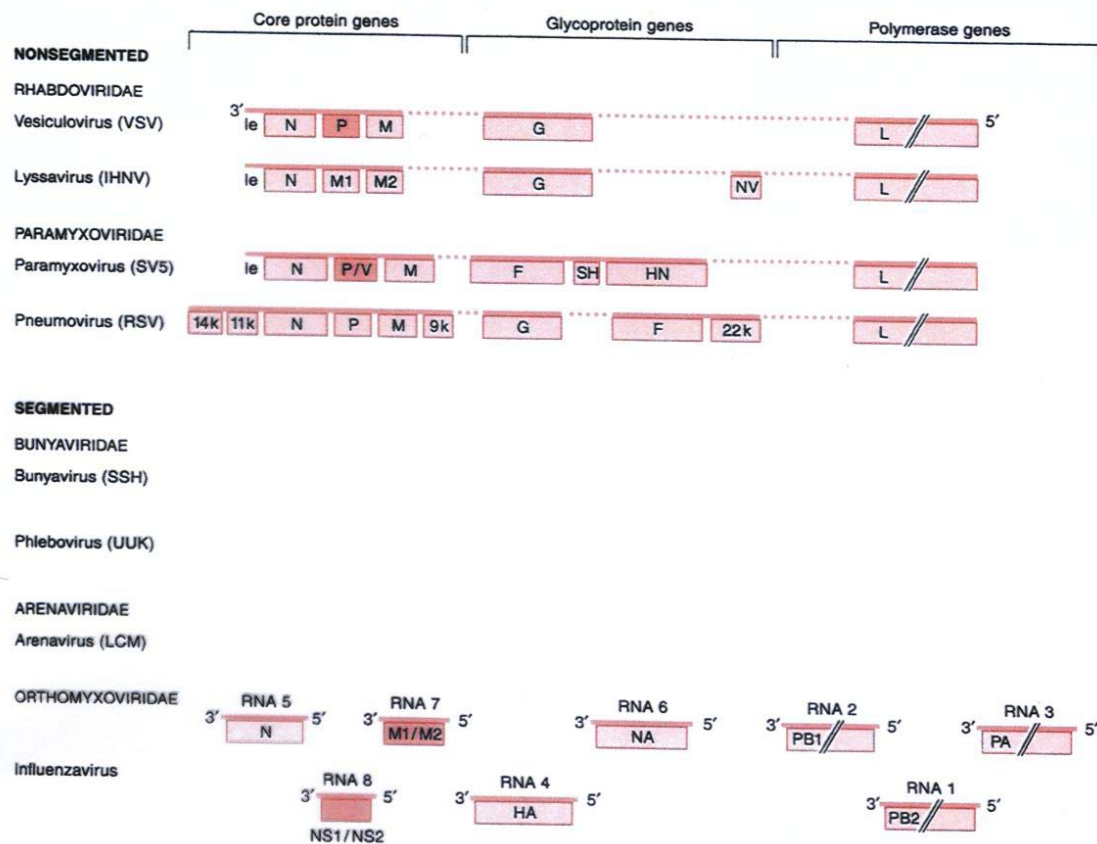
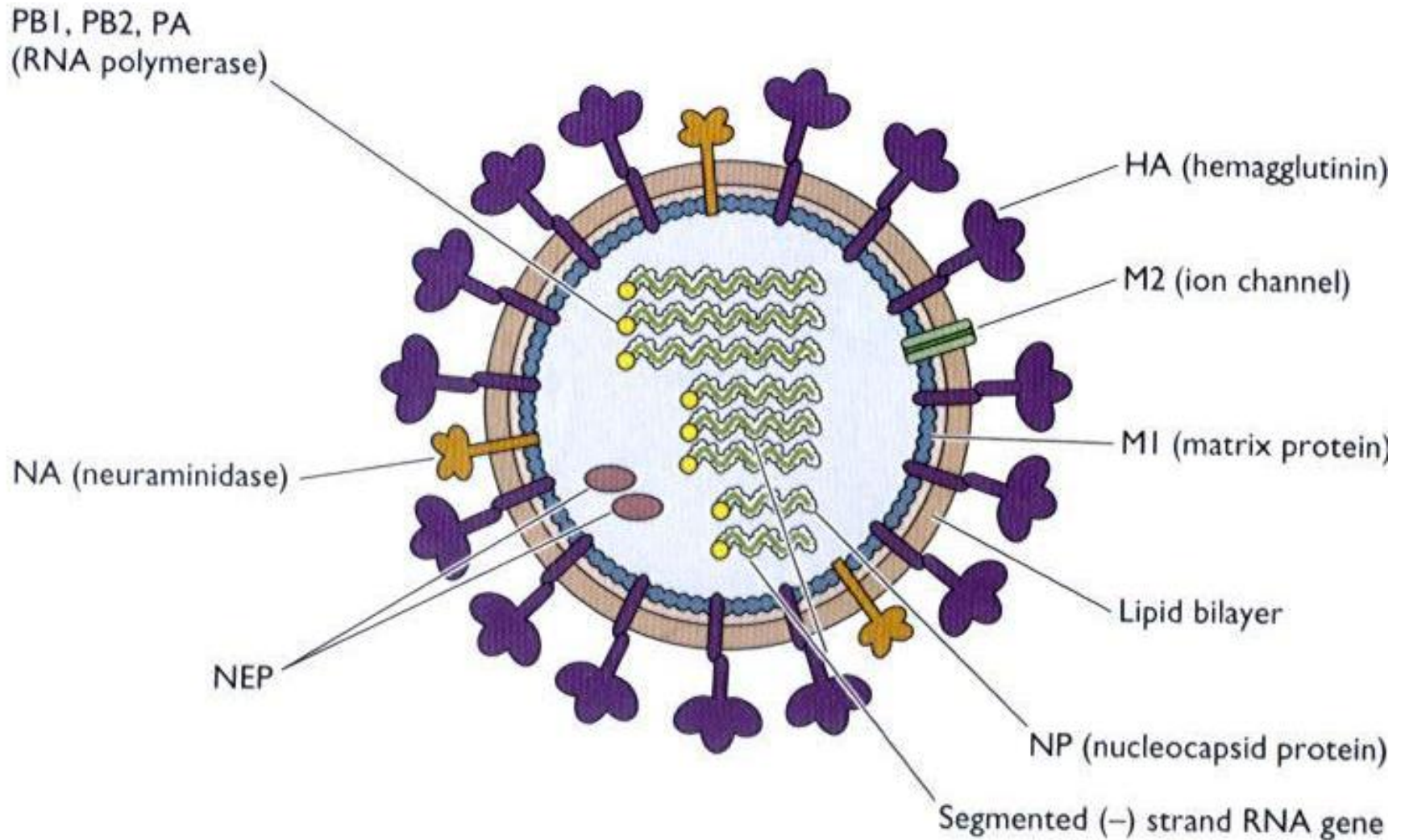


Figure 2.17

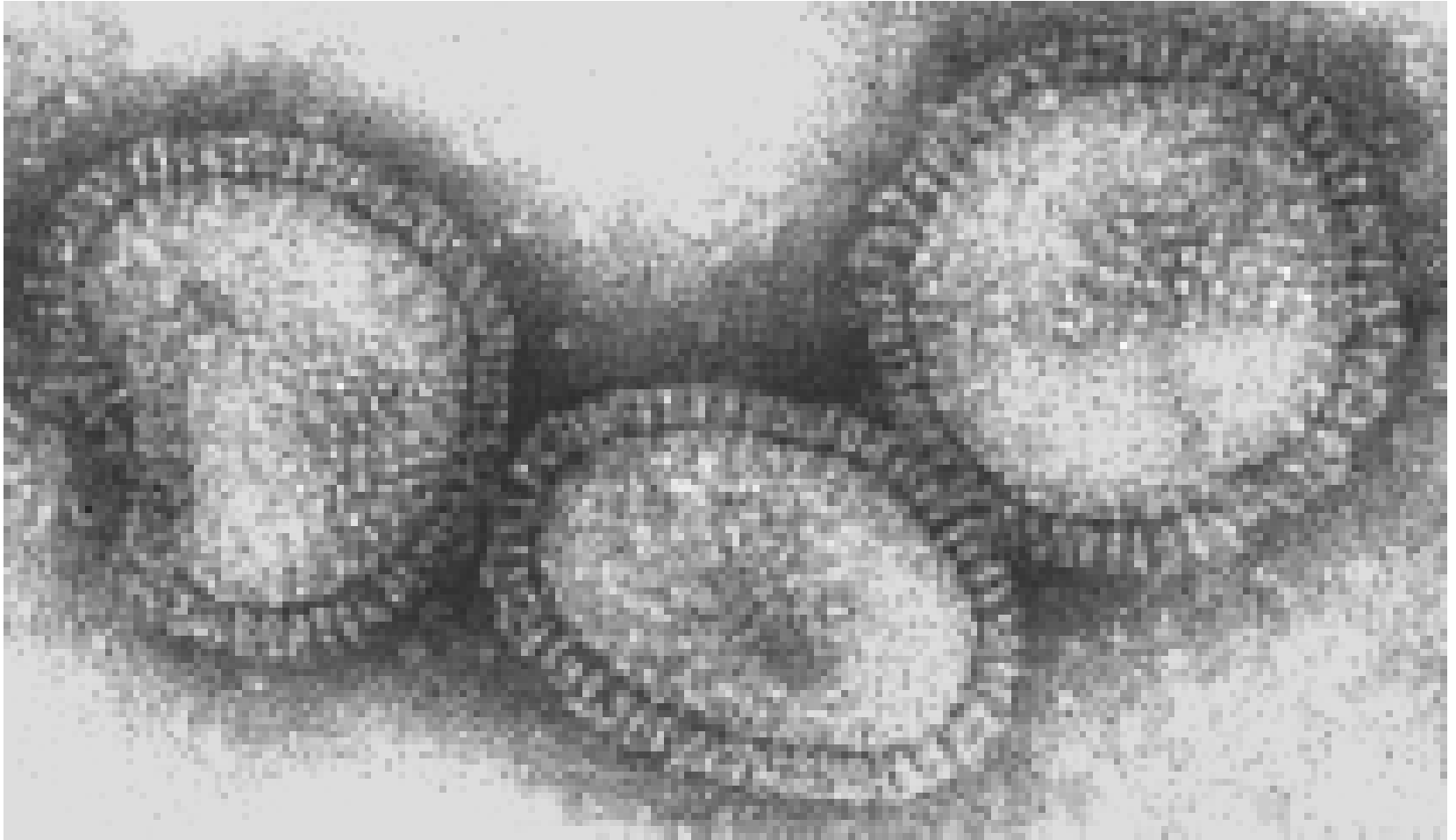
Comparative genome organizations of minus-strand viruses. The maps are only roughly to scale. Genes (boxes) are aligned to indicate similarities of genome organization. Genes shaded in darker color encode more than one protein in overlapping ORFs; genes with diagonal hatching are transcribed from vcRNA (ambisense). For vesiculovirus (VSV) the proteins encoded are N, the nucleocapsid protein, P, the phosphoprotein, M, the matrix protein, G, the glycoprotein, and L, the replicase (the large protein). The leaders (le) are short sequences that transcribed but not translated. For lyssavirus (IHNV), M1 is a phosphoprotein corresponding to P, M2 corresponds to M, and there is a small nonvirion protein called NV. Among the Paramyxoviridae, N is the nucleocapsid protein, P the phosphoprotein, M the matrix protein, F the fusion protein (a glycoprotein), and L the replicase. Paramyxovirus (SV5), contains HN, the hemagglutinin-neuraminidase protein (a glycoprotein) and a small, hydrophobic nonstructural protein (SH) located between F and HN. For pneumovirus (RSV) several additional genes are shown, including glycoprotein G. The genomes of the segmented viruses have been aligned such that segments are shown below their functional counterparts in the nonsegmented viruses.

Bunyaviruses and arenaviruses encode the nucleocapsid protein N, two envelope glycoproteins G1 and G2, and the replicase L. Some also encode various NS or nonstructural proteins. The proteins encoded in the eight segments of influenza virus are the nucleoprotein N, the phosphorylated proteins NS1 (and NS2 and M2), the matrix protein M1, the hemagglutinin HA, the neuraminidase NA, and the three replicase components, PB1, PB2, and PA.

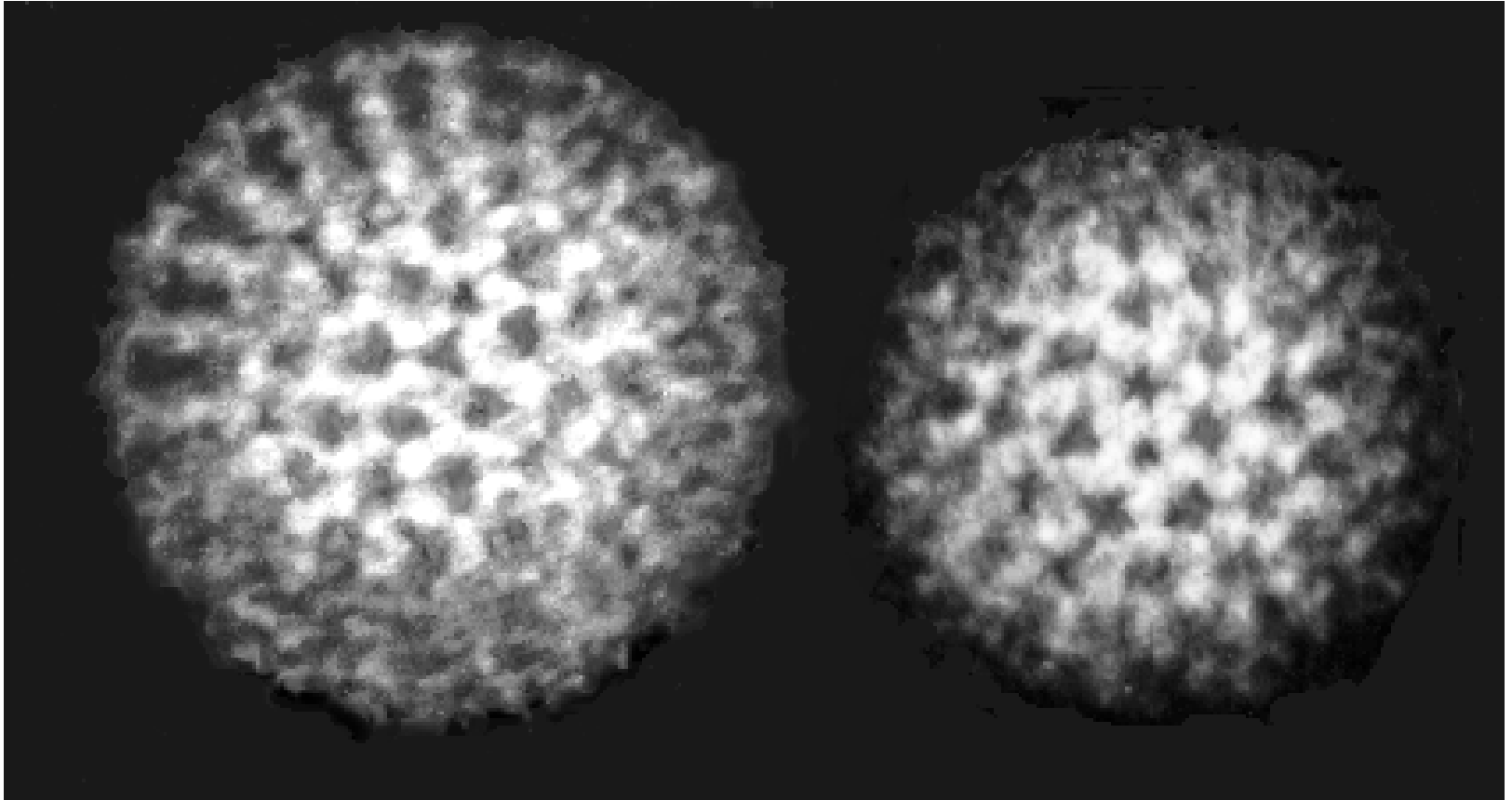
Orthomyxoviridae



Orthomyxoviridae



Orthomyxoviridae



Vírus Influenza

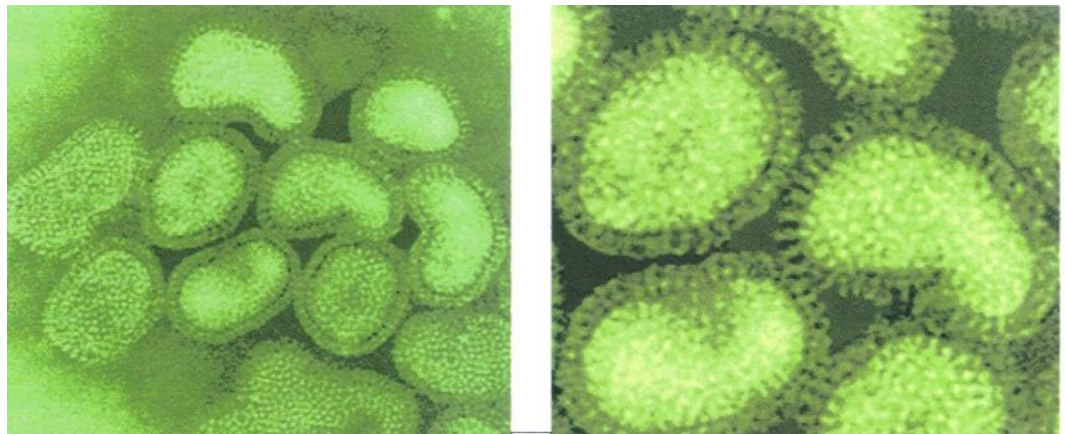
Três géneros (tipos): **A**, B, C

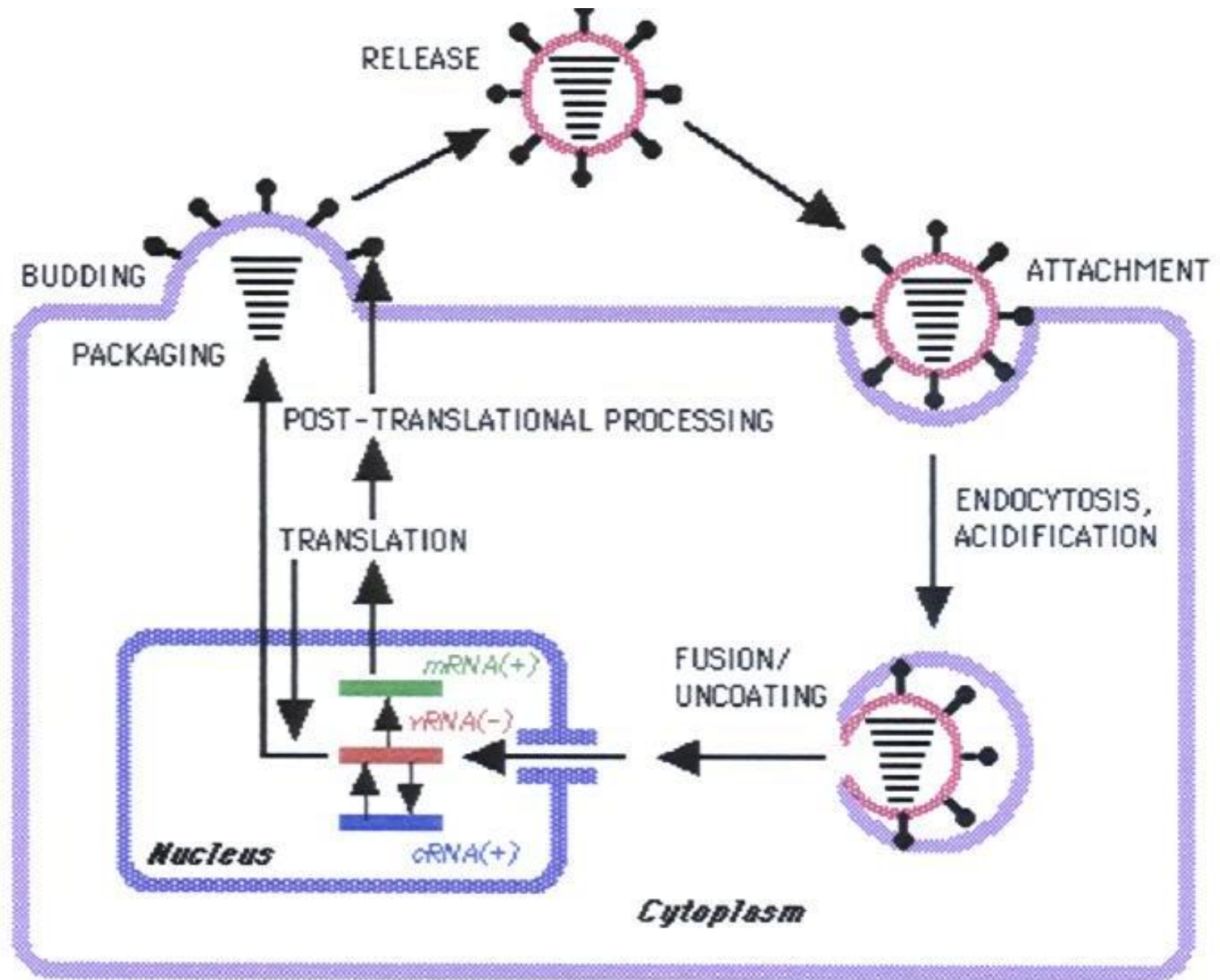
Apenas o tipo **A** apresenta um largo espectro de hospedeiros

Vírus Influenza A

Subtipos definidos pelos antígenos de superfície **H** (hemaglutinina) e **N** (neuraminidase):

15 subtipos **H** e 9 subtipos **N**

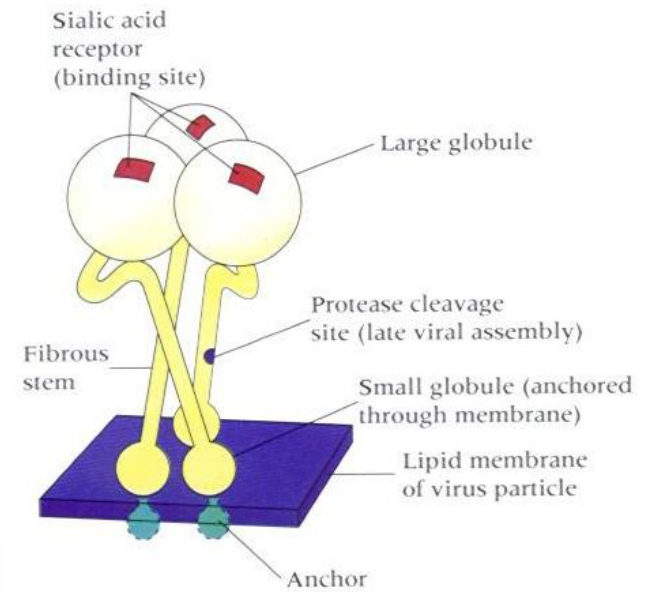




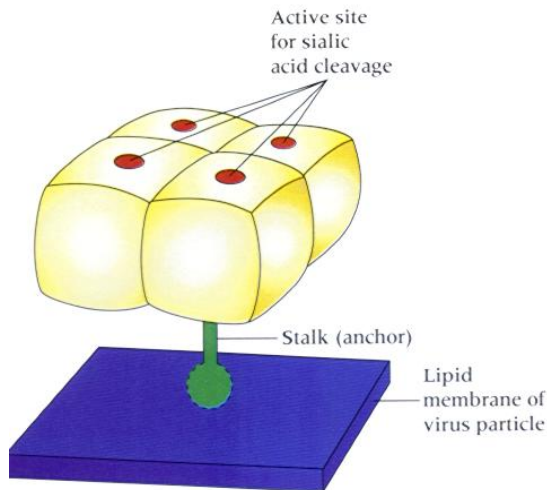
**Hemagglutinin and Neuraminidase
Subtypes of Influenza A Virus**

Subtype	Species of origin			
	Human	Swine	Horse	Bird species
H1	PR/8/34	Sw/Ia/15/30		Dk/Alb/35/76
H2	Sing/1/57			Dk/Ger/1215/73
H3	HK/1/68	Sw/Taiwan/70	Eq/Miami/1/63	Dk/Ukr/1/63
H4				Dk/Cz/56
H5				Tern/S.A./61
H6				Ty/Mass/3740/65
H7			Eq/Prague/1/56	FPV/Dutch/27
H8				Ty/Ont/6118/68
H9				Ty/Wis/1/66
H10				Ck/Ger/N/49
H11				Dk/Eng/56
H12				Dk/Alb/60/76
H13				Gull/Md/704/77
H14				Mall/Gurjev/ 263/82
N1	PR/8/34	Sw/Ia/15/30		Ck/Scot/59
N2	Sing/1/57	Sw/Taiwan/70		Ty/Mass/3740/65
N3				Tern/S.A./61
N4				Ty/Ont/6118/68
N5				Sh/Austral/1/72
N6				Dk/Cz/56
N7			Eq/Prague/1/56	FPV/Dutch/27
N8			Eq/Miami/1/63	Dk/Ukr/1/63
N9				Dk/Mem/546/74

HA



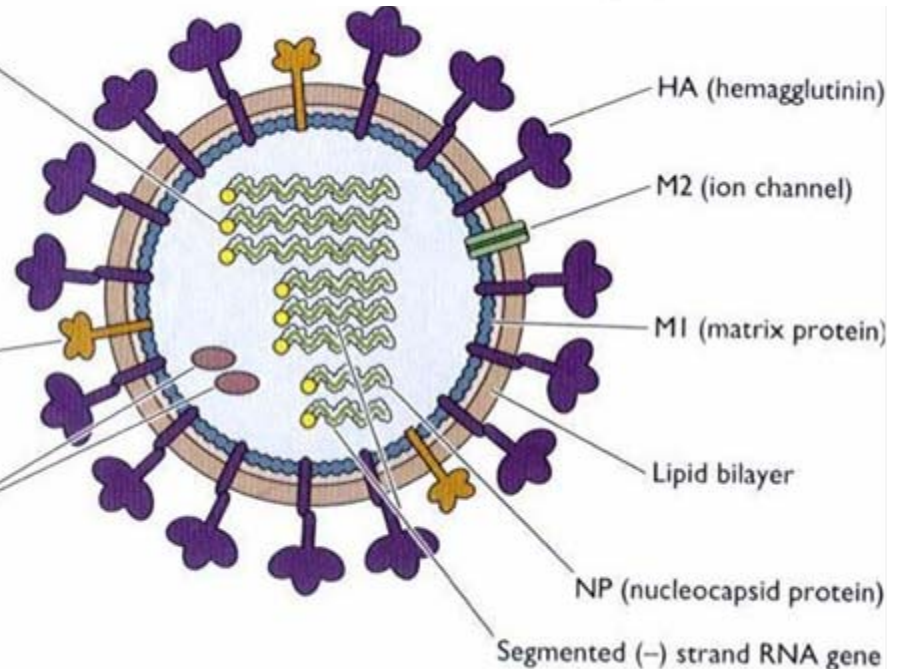
NA



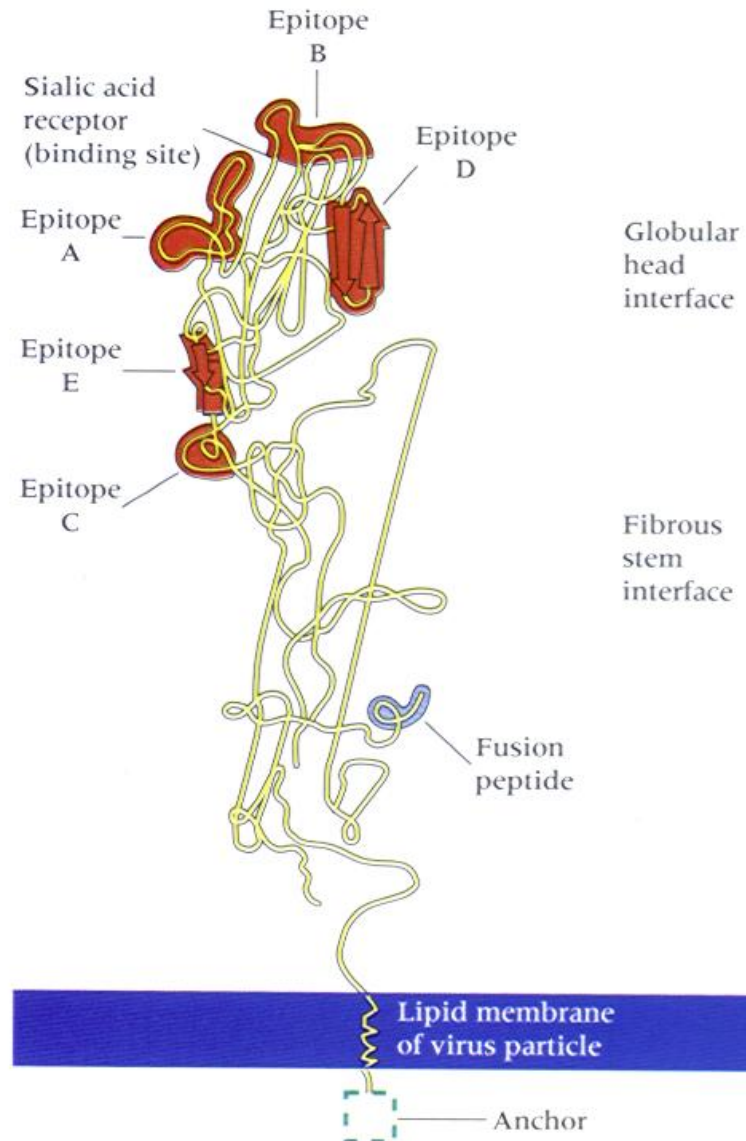
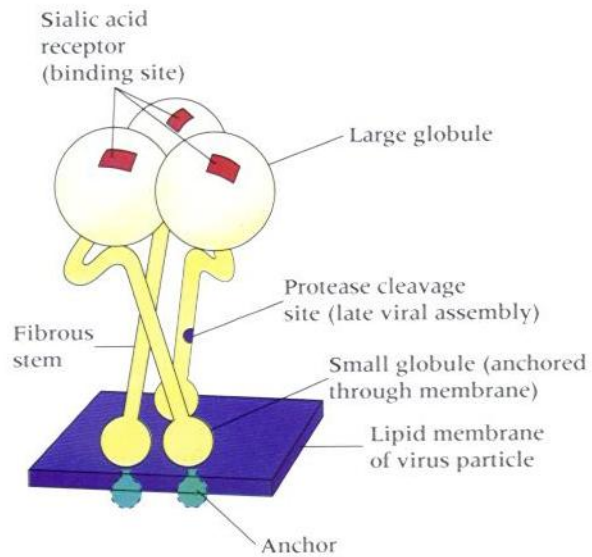
PB1, PB2, PA
(RNA polymerase)

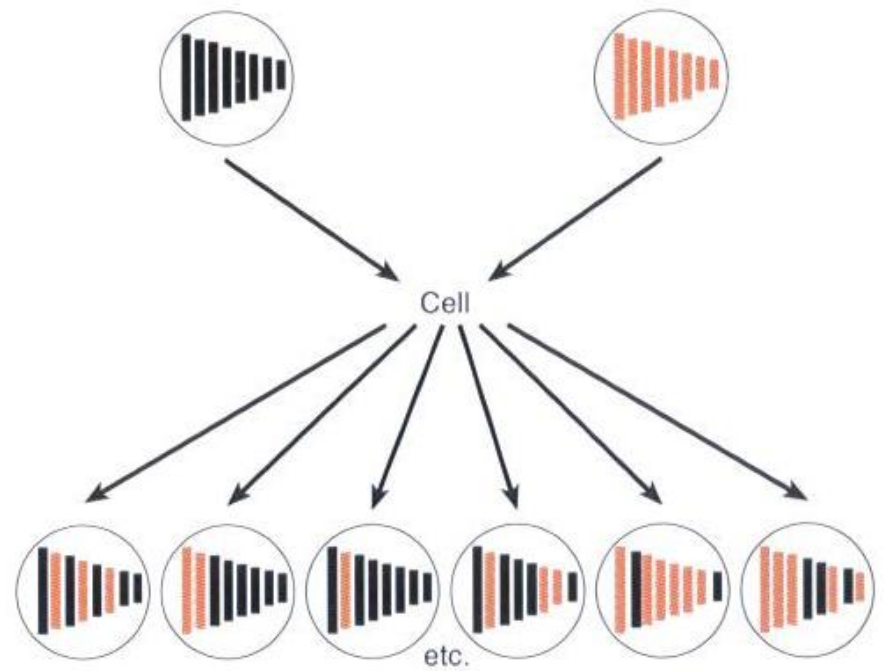
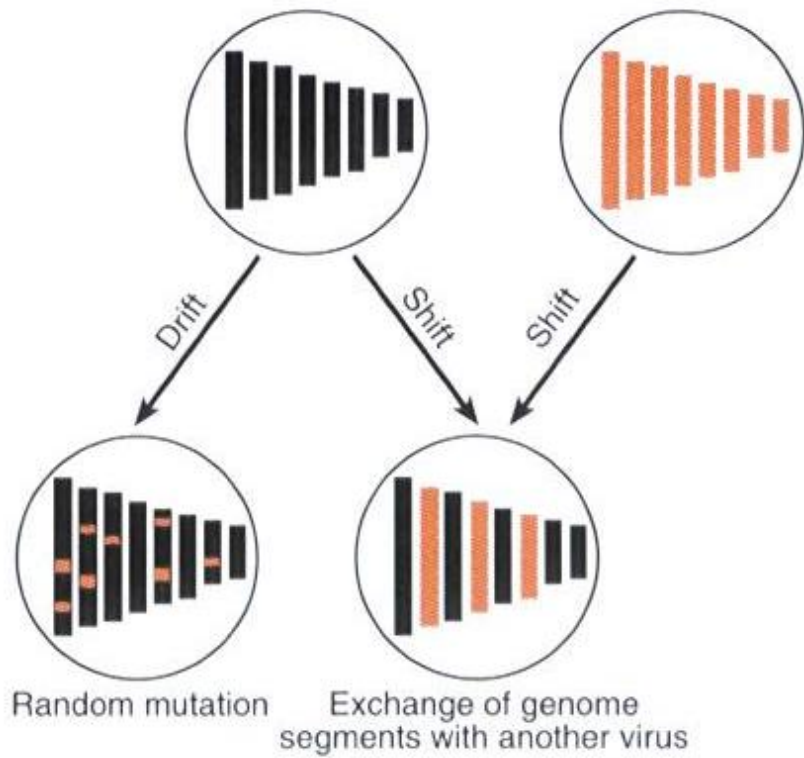
NA (neuraminidase)

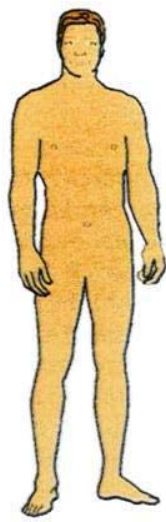
NEP



HA





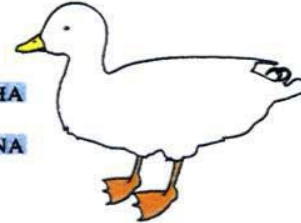


Influenza A
chromosome number
(human virus)

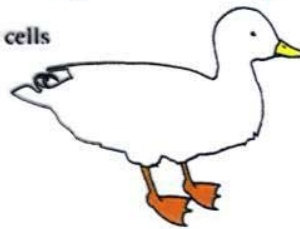
1 hu	
2 hu	
3 hu	
4 hu	→ HA
5 hu	
6 hu	→ NA
7 hu	
8 hu	

Influenza A
chromosome number
(duck virus)

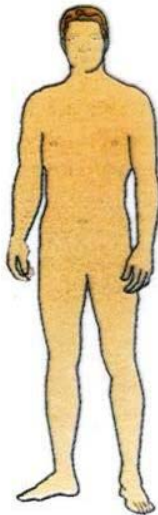
1 dk	
2 dk	
3 dk	
4 dk	→ HA
5 dk	
6 dk	→ NA
7 dk	
8 dk	



Infection of the same cells
by human and duck
influenza A viruses



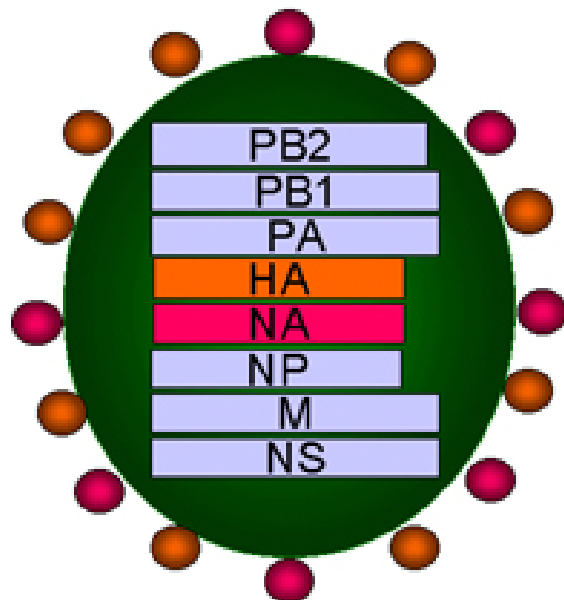
Progeny influenza A viruses can
contain up to 256 combinations
of different chromosomes from the
human or duck viral RNA genomes



An example of an influenza A virus selected
for growth in nonimmunized humans
(pandemic strain)

1 hu	
2 hu	
3 hu	
4 dk	→ HA
5 hu	
6 dk	→ NA
7 hu	
8 hu	

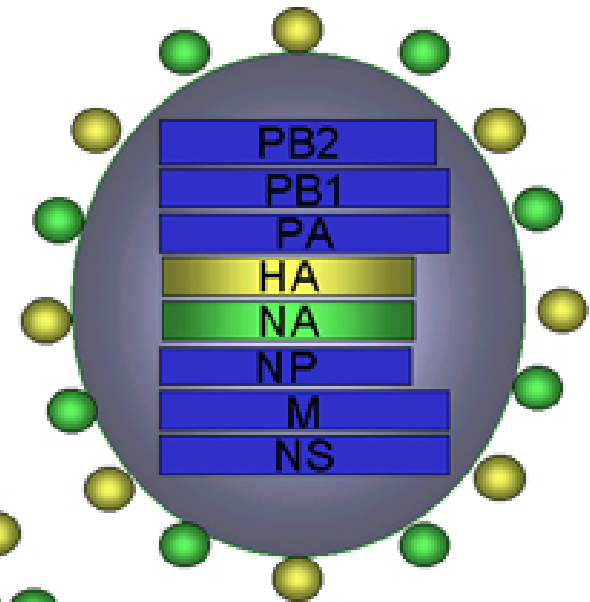
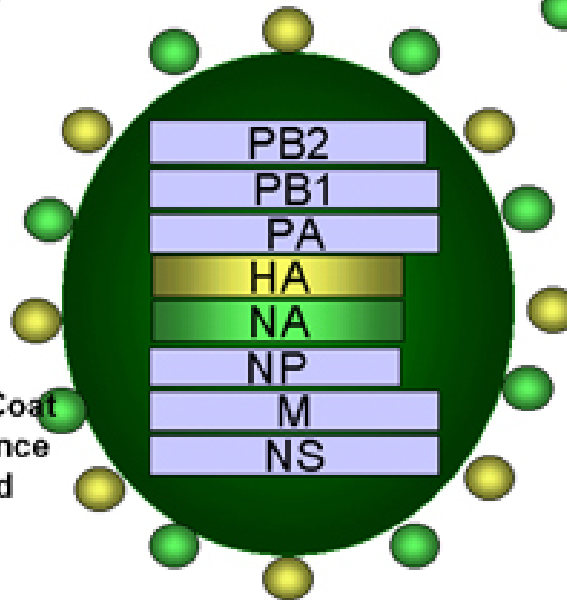
Infection of one cell with both human and duck influenza A viruses can result in a reassortment of viral chromosomes, followed by selection for a virulent progeny virus not previously present in the human population. Animal reservoirs, which harbor influenza A virus with distinct chromosomes producing a wide variety of HA and NA antigenic subunits, are the source for new viral genes.



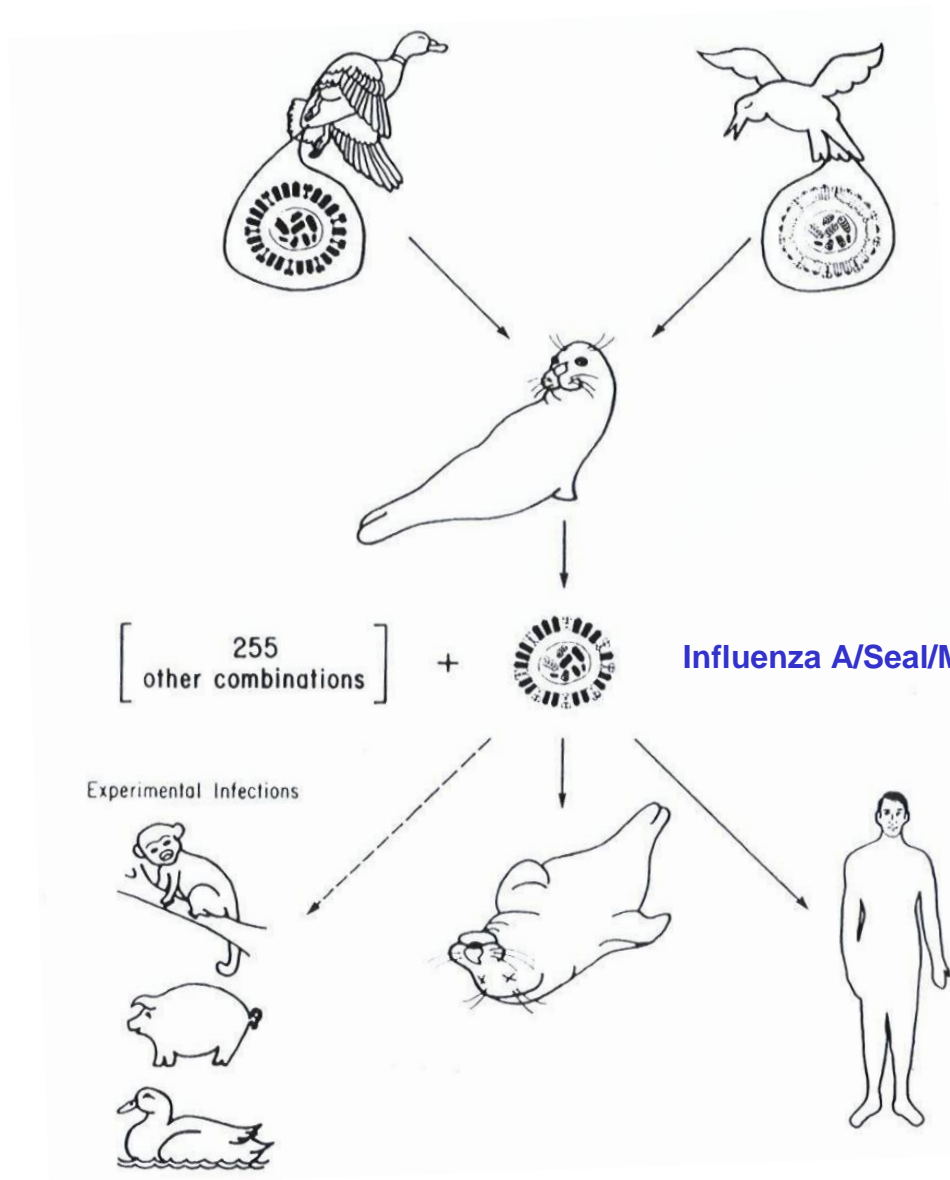
Attenuated Donor
Master Strain

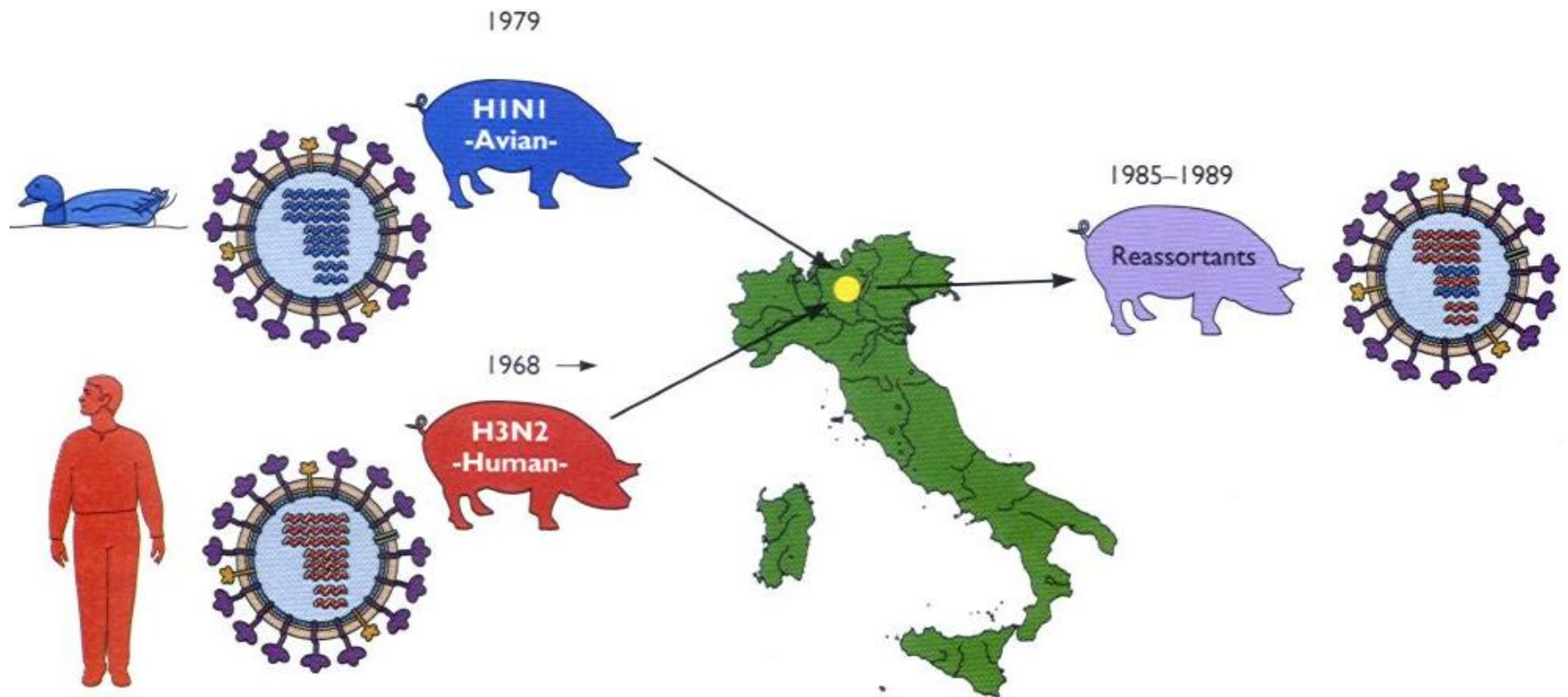
Attenuated Vaccine Strain: Coat
of Virulent strain with Virulence
Characteristics of Attenuated
Strain

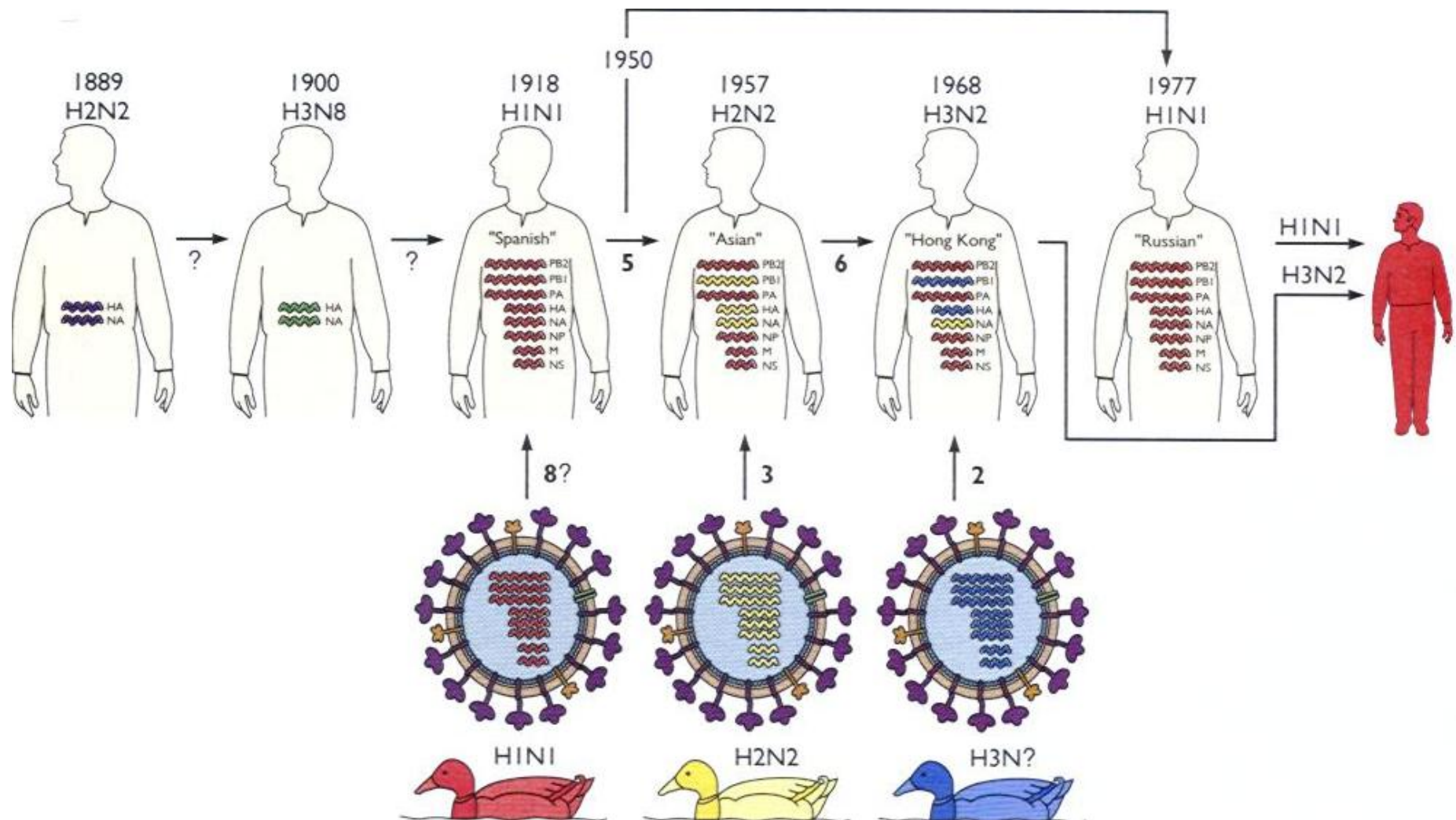
X



New Virulent
Antigenic Variant
Strain







1933

Isolamento do vírus em humanos

1935

Primeira vacina testada

1937

Produção da vacina

1940-44

Ensaio em grande escala da vacina

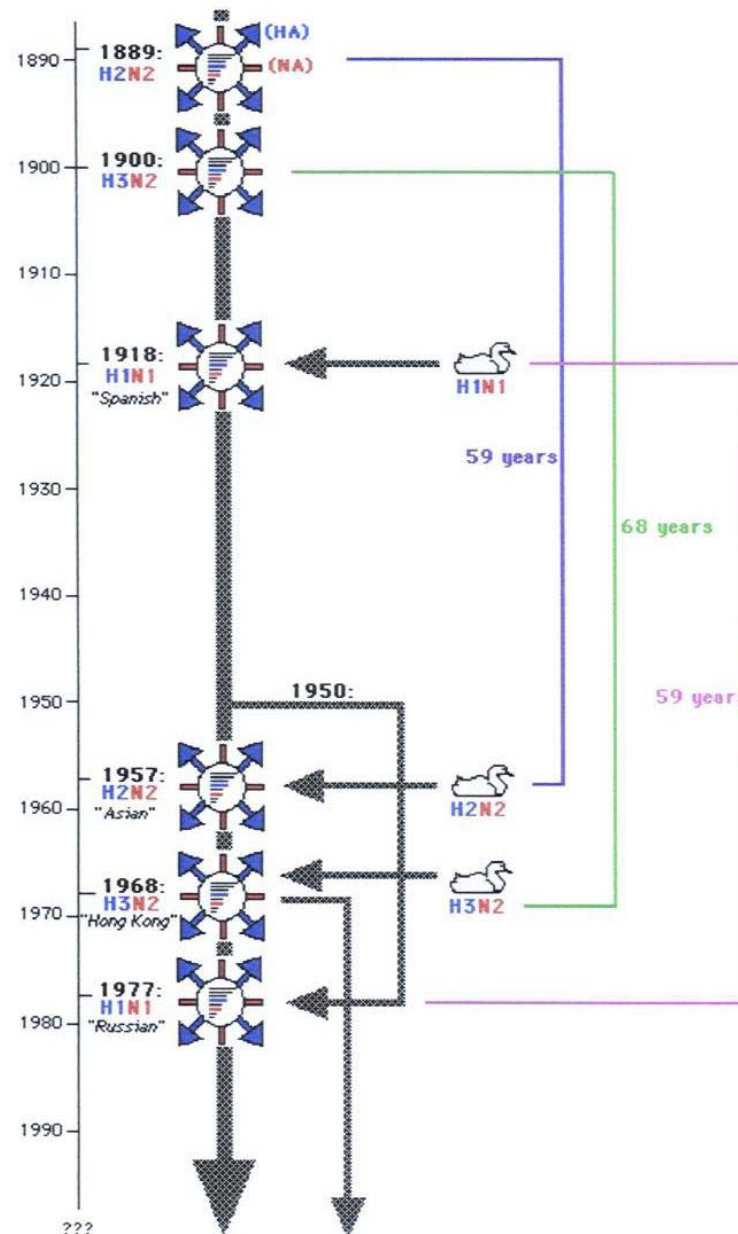
1948-49

Vacinação em massa

1968-69

Purificação da vacina

Hemaglutinina e Neuraminidase são caracterizadas



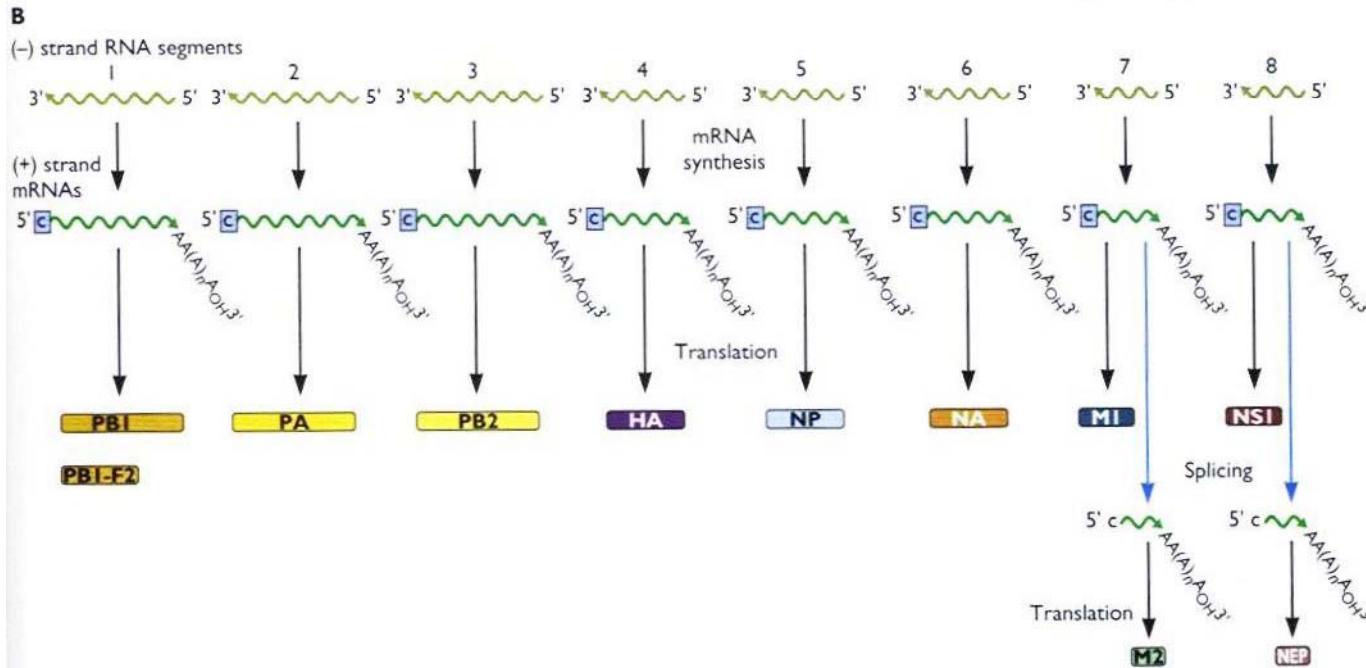
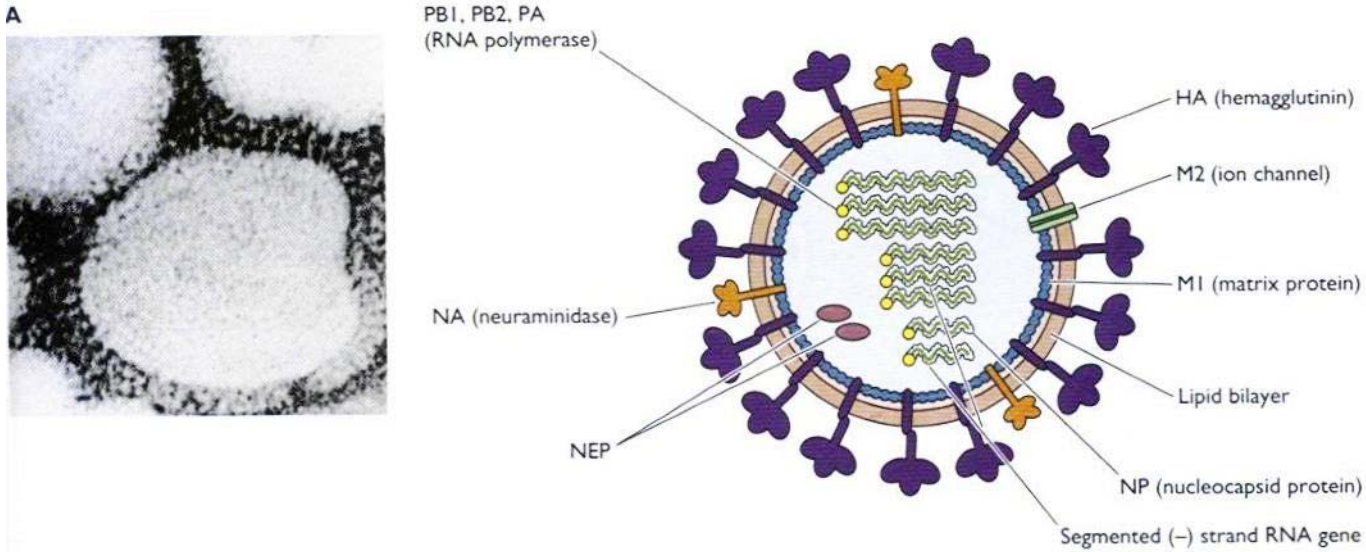


Figure 8 Structure and genomic organization of the orthomyxovirus influenza A virus

Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

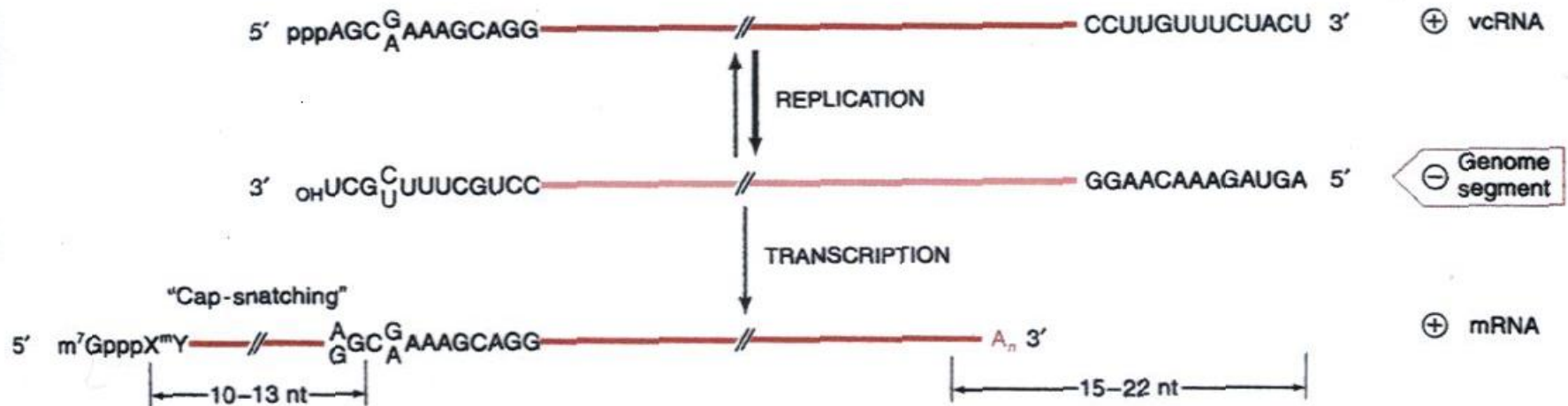
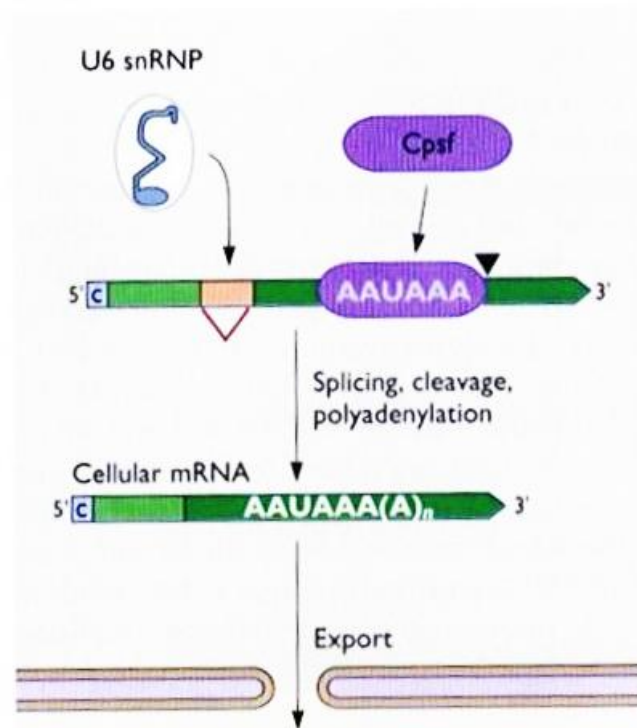


Figure 2.19

Relationship between genome RNAs, mRNAs, and vcRNA of orthomyxoviruses (influenza). The figure illustrates the nonidentity between the mRNA and the vcRNA for one representative genome segment. Plus-strand mRNAs have caps and leader sequences derived from cellular mRNAs (shown in Figure 2.18) and terminate with a poly A tail. Plus-strand templates (vcRNAs) are exact complements of the genomic minus-strand RNAs.

-NSI



+NSI

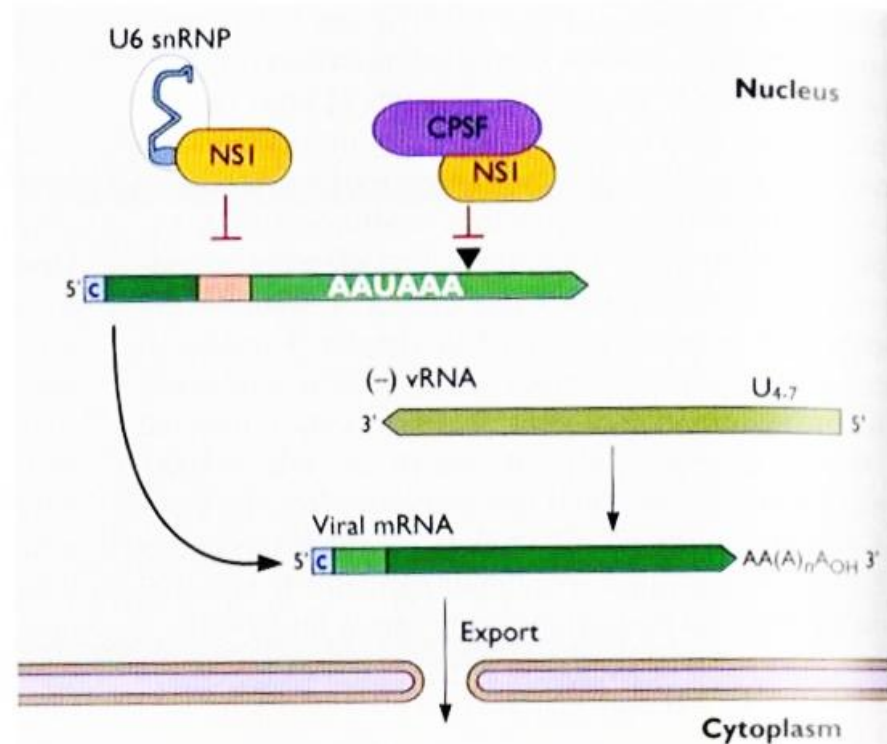


Figure 10.19 Model of inhibition of cellular pre-mRNA processing by the influenza A virus NSI protein. The viral protein binds to a subunit of the cleavage and polyadenylation specificity protein (Cpsf). As indicated (right), this interaction prevents binding of cleavage and polyadenylation specificity protein to the 5'AAUAAA3' polyadenylation signals at the poly(A) addition sites (black arrow-head) of cellular pre-mRNAs and formation of the 3' ends of mature mRNAs. In the absence of 3'-end cleavage, the pre-mRNA probably remains associated with the transcriptional machinery and DNA template and is not available for export to the cytoplasm. Consequently, the intranuclear concentration of capped cellular pre-mRNAs from which the primers for viral mRNA synthesis are acquired would be increased. The viral NSI protein can also bind to U6 snRNA to inhibit splicing of cellular pre-mRNAs.

Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

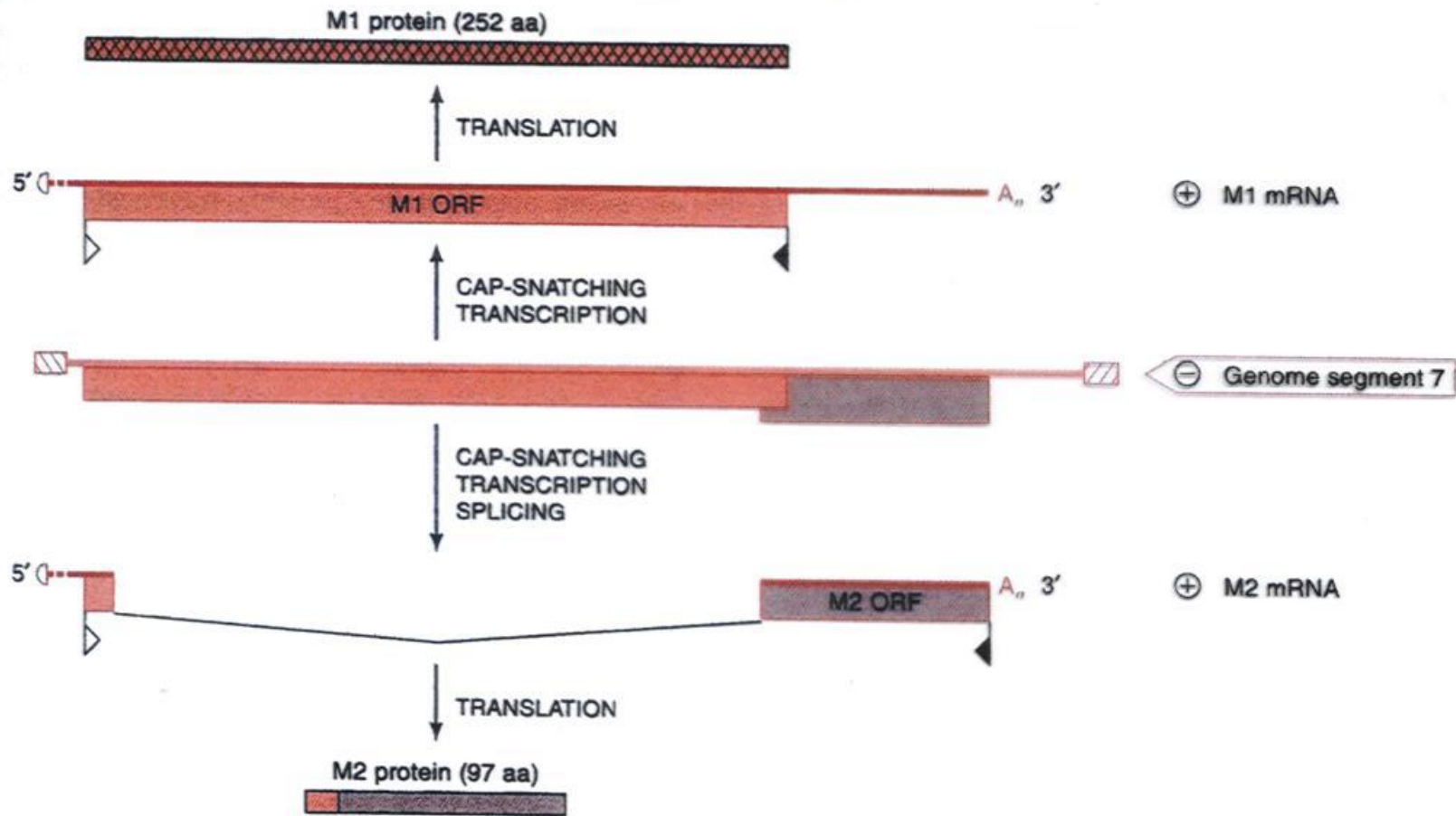


Figure 2.21

Transcription and translation strategies for influenza virus segment 7. The M1 mRNA is synthesized as shown in Figure 2.19. The M2 mRNA is made by splicing a short sequence from the 5' end of the M1 ORF (indicated throughout this figure by colored boxes) to sequences containing a second ORF. Both mRNAs have caps (open symbols) derived from cellular capped messages ("cap-snatching"). Diagonally patterned boxes at the termini of the genome segment indicate short self-complementary sequences that are absent from the mRNAs.

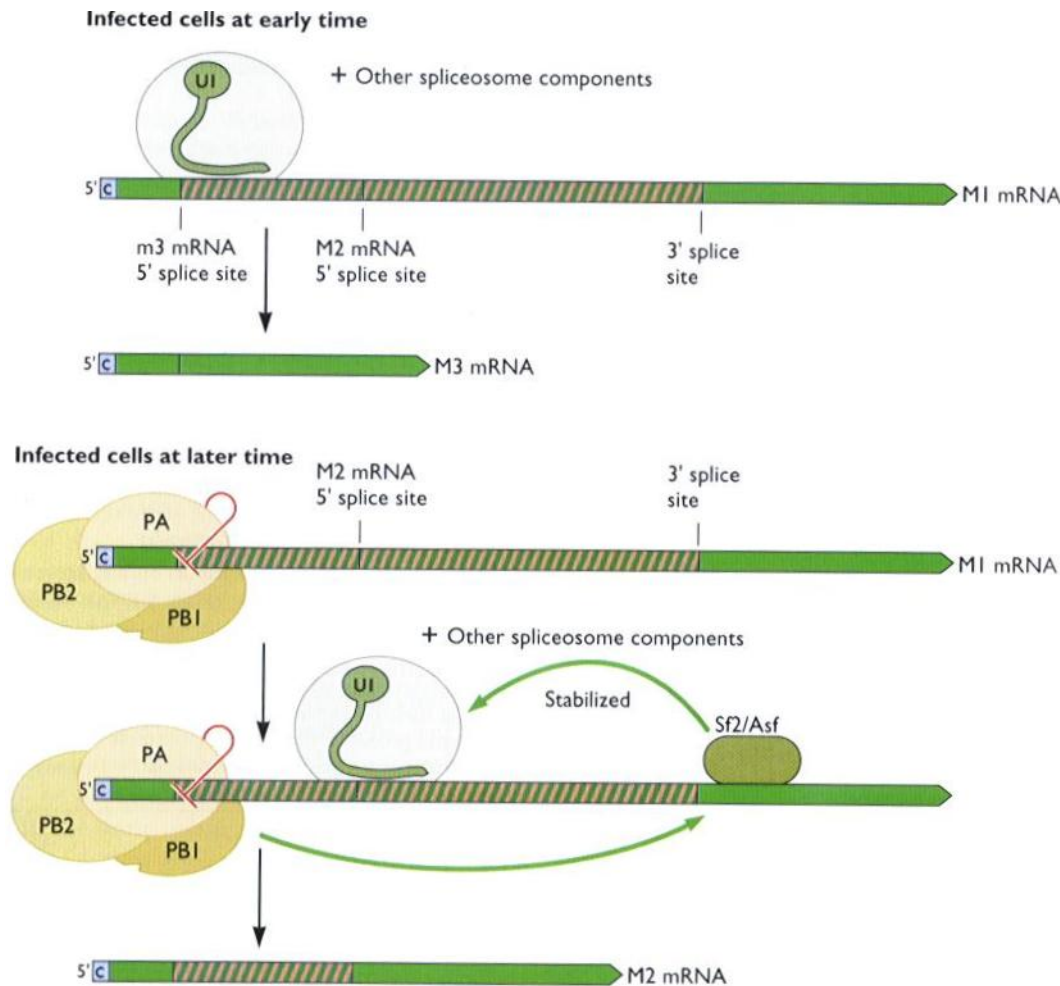
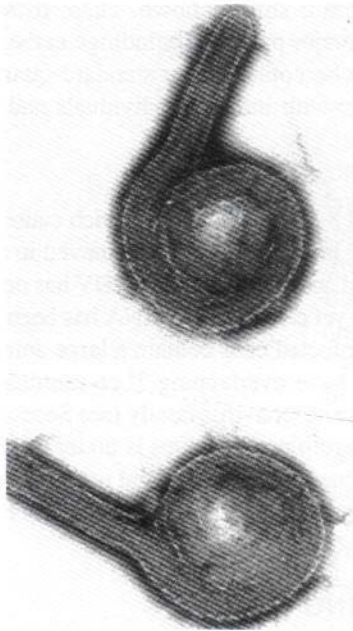


Figure 10.18 Model of regulation of splicing of the influenza A virus M1 mRNA. The mRNA synthesized from the M genomic RNA segment encodes the M1 protein but can also be spliced to produce the mRNA specifying the M2 protein. However, at early times of infection (top), before the synthesis of P proteins, the strong 5' splice site for a third RNA, m3 mRNA, is used exclusively and only m3 mRNA is made. At later times (below), the P proteins bind to a specific sequence near the 5' end of the influenza A virus mRNAs. In the case of the M1 mRNA, such binding blocks access to the m3 mRNA 5' splice site, allowing unspliced M1 mRNA to be produced in infected cells. The 5' splice site for the M2 mRNA is suboptimal, and its recognition depends on binding of the cellular SR protein splicing factor 2 (Sf2/Asf) to a purine-rich splicing enhancer in the 3' exon of M1 mRNA. Such binding requires interaction of the P proteins with the 5' end of M1 mRNA, suggesting that the conformation of the RNA is altered upon binding of P proteins.

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(a)



(b)

(c)

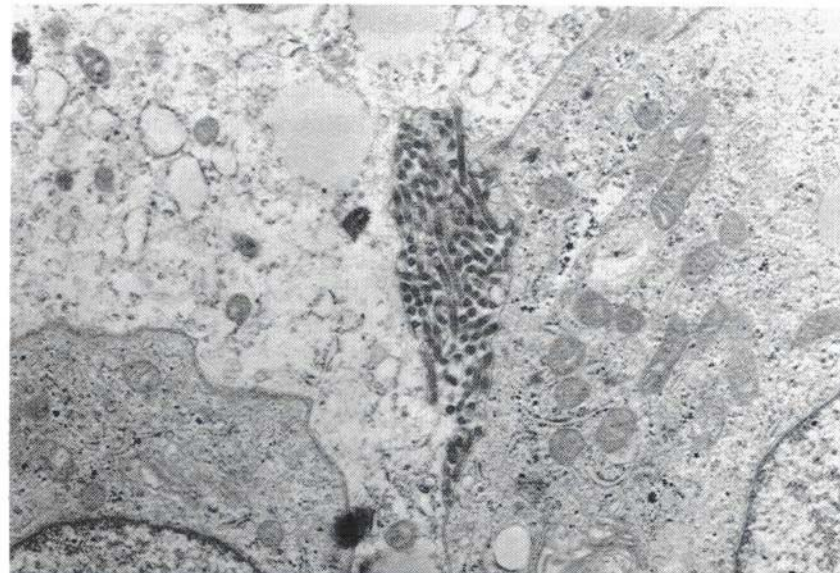


FIGURE 4.18

(a) Ebola virus passed in monkey cells showing typical 6-shaped particles by negative stain ($\times 73,500$). (b) Ebola virus isolated in 1976 on monkey Vero cells. Negative stain ($\times 70,000$). This photo is actually the first Ebola virus ever visualized. (c) Marburg virus passed from a human case (liver isolate) through monkey Vero cells. Virus particles are observed between the spaces of two cells ($\times 32,200$). (Courtesy of F. A. Murphy)