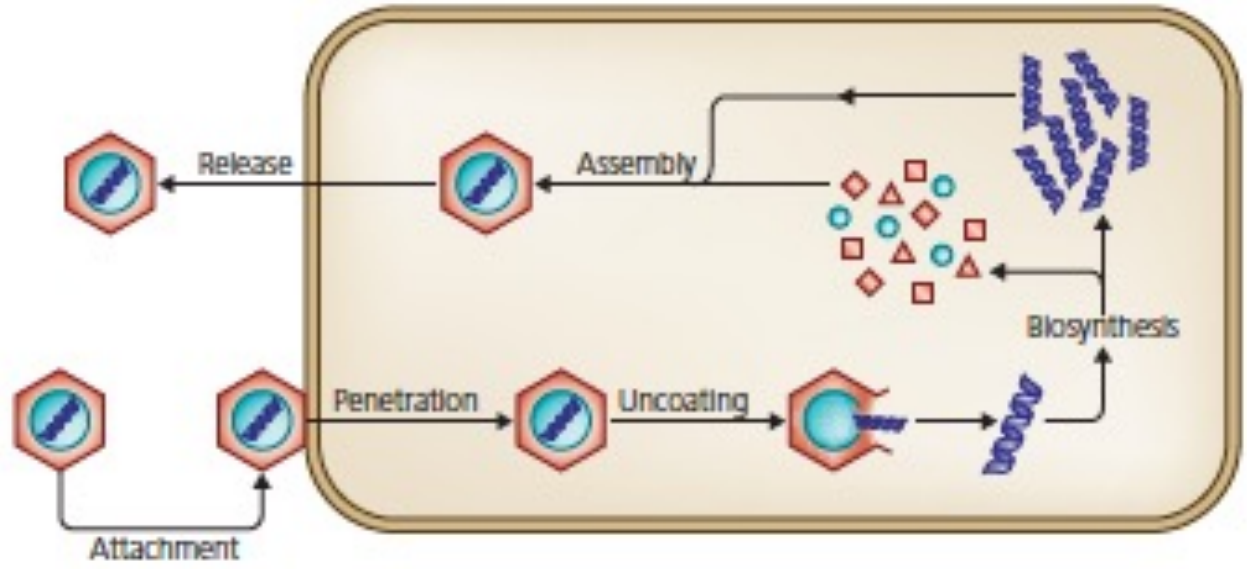
Virus Multiplication

Viruses multiply by assembling many progeny particles from a pool of virus specified components, whereas cells multiply by binary fission.



Introduction to Modern Virology, Seventh Edition. N. J. Dimmock, A. J. Easton and K. N. Leppard.

Virus Multiplication

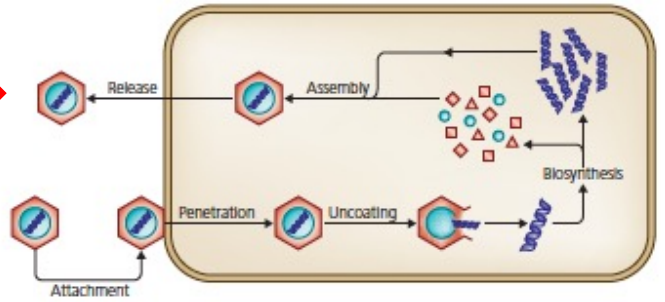
The efficiency of multiplication demonstrated by viruses is such that the infection of a single host can generate more new viruses than there are individuals in the host population. For example, a single human infected with influenza virus can shed sufficient virus particles to be theoretically capable of infecting the entire human population.

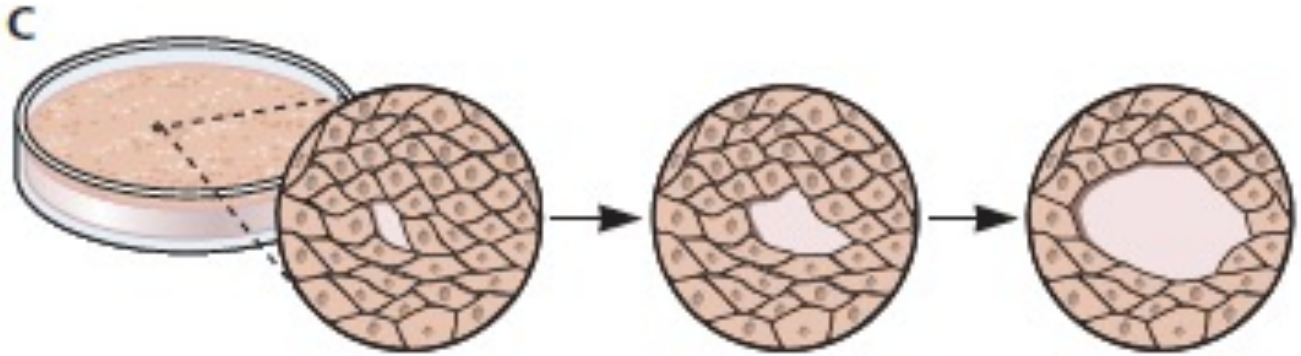
Particle-to-PFU ratios of some animal viruses

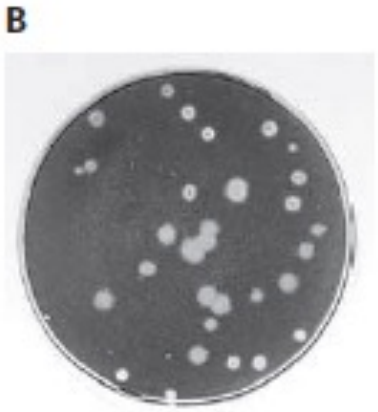
Virus	Particle/PFU ratio
Papillomaviridae	
Papillomavirus	10,000
Picornaviridae	
Poliovirus	30–1,000
Herpesviridae	
Herpes simplex virus	50–200
Polyomaviridae	
Polyomavirus	38–50
Simian virus 40 100–200	
Adenoviridae	20–100
Poxviridae	1–100
Orthomyxoviridae	
Influenza virus	20–50
Reoviridae	
Reovirus	10
Alphaviridae	
Semliki Forest virus	1–2

Counting virus infectious particles by the plaque assay

Many, many new particles

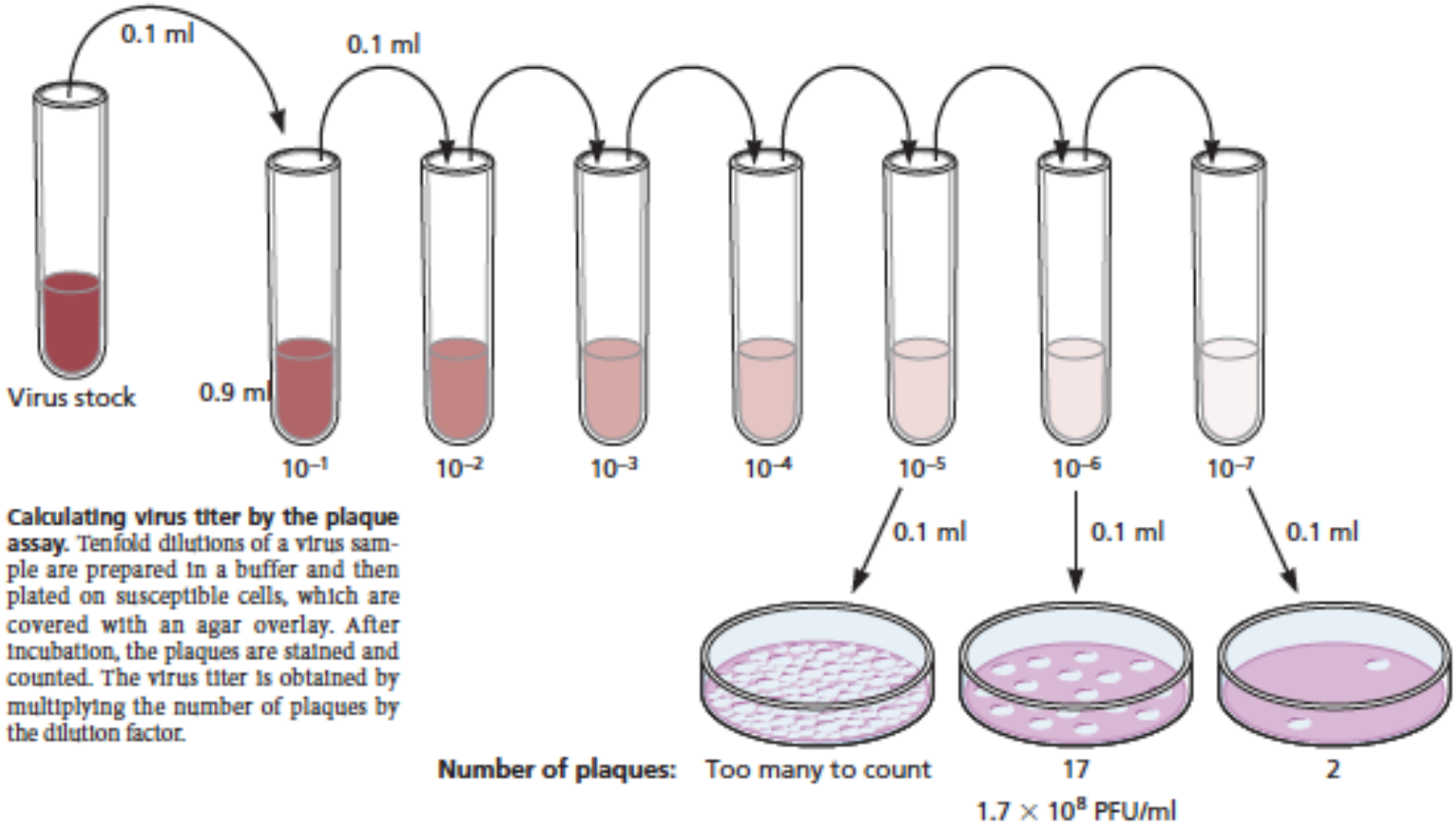






From Flint et al. *Principles of Virology* (2015), ASM Press

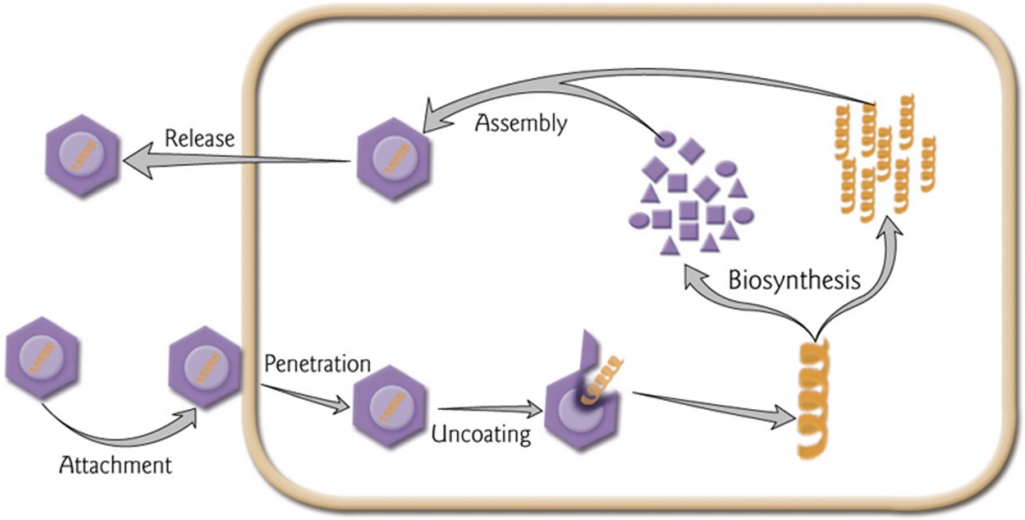
Calculating virus titre by the plaque assay



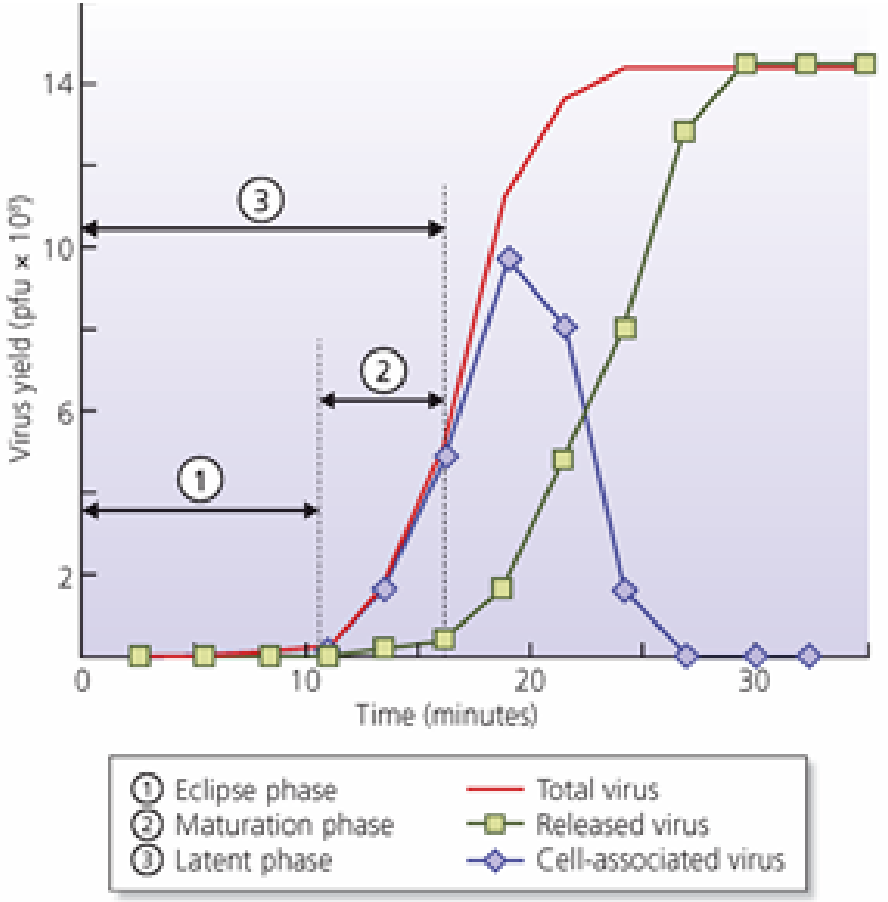
Calculating virus titer by the plaque assay. Tenfold dilutions of a virus sample are prepared in a buffer and then plated on susceptible cells, which are covered with an agar overlay. After incubation, the plaques are stained and counted. The virus titer is obtained by multiplying the number of plaques by the dilution factor.

From Flint et al. *Principles of Virology* (2015), ASM Press

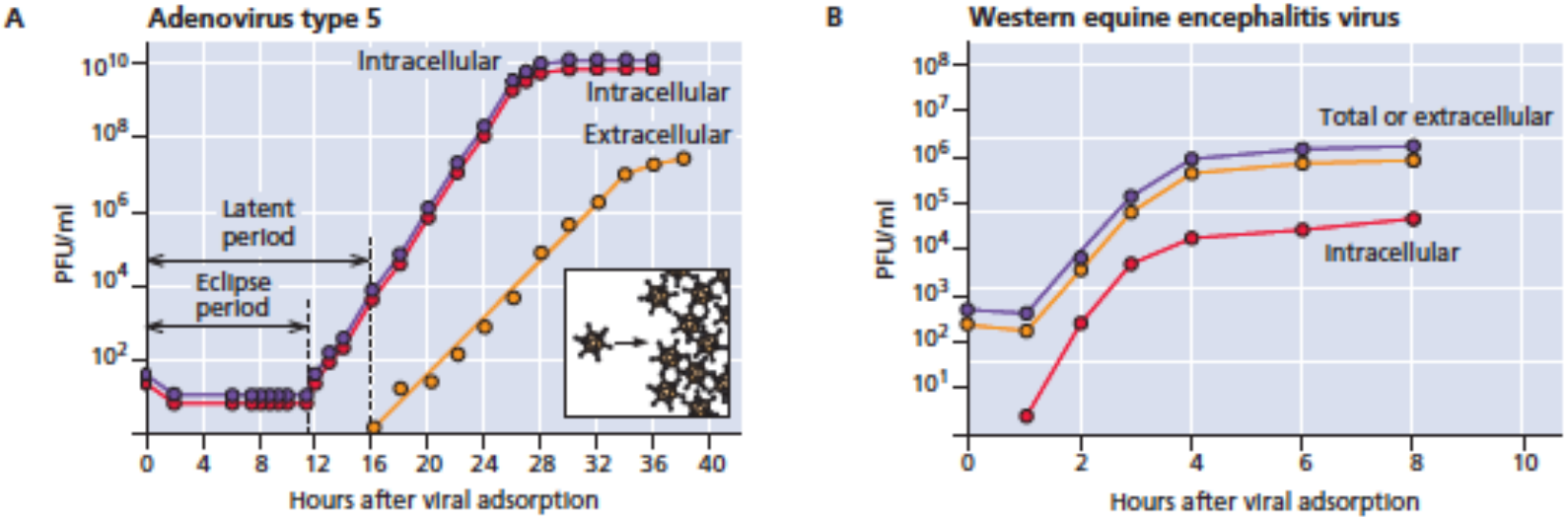
One-Step Growth Curve
Ellis and Delbruck (1939)



Viral genome drives the formation of new viral particles which are the result of viral component assembly



One-Step Growth Curve of animal viruses

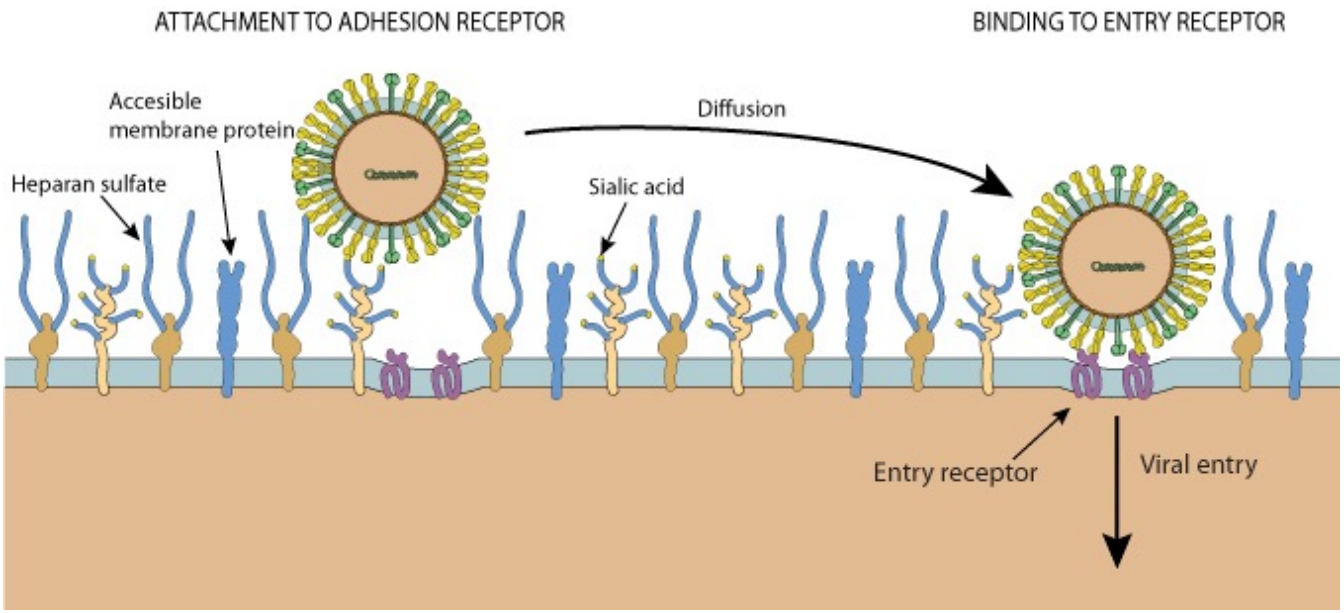


The kinetics of the one-step growth curve can vary dramatically among different viruses. For example, enveloped viruses that mature by budding from the plasma membrane generally become infectious only as they leave the cell, and therefore little intracellular infectious virus can be detected (Fig. B). The curve shown in Fig. A illustrates the pattern observed for a DNA virus with the long latent and synthetic phases typical of many DNA viruses, some retroviruses, and reovirus. For small RNA viruses, the entire growth curve is complete within 6 to 8 h, and the latent and synthetic phases are correspondingly shorter.

One-step growth curve analysis can provide quantitative information about different virus-host systems. It is frequently employed to study mutant viruses to determine what parts of the infectious cycle are affected by a particular genetic lesion. It is also valuable for studying the multiplication of a new virus or viral replication in a new virus-host cell combination.

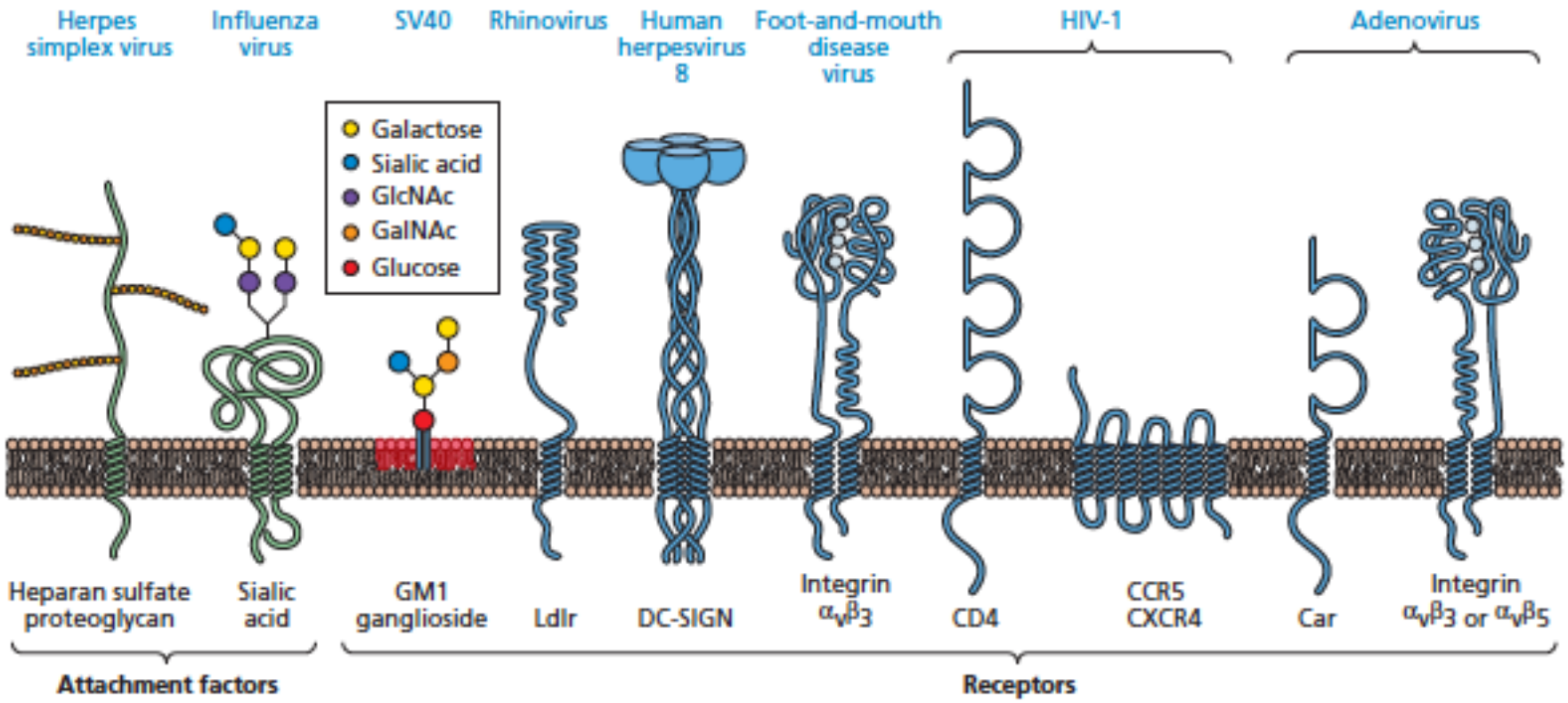
From Flint et al. *Principles of Virology* (2015), ASM Press

Viral attachment to host cell



Cell receptors to virus can be classified in two classes: adhesion receptors are attaching the virus in a reversible manner to target cells or organs. This adhesion is not mandatory for virus entry, and alone do not trigger entry. Nonetheless it enhances significantly infectivity by concentrating the virus in the vicinity of it's entry receptors. These receptors are triggering virus entry by endocytosis/pinocytosis or by inducing fusion/penetration, and the consequences of this binding are irreversible. Entry receptors are often difficult to access for the virion, which circumvents this problem by binding first to adhesion receptors, which increases the probability of binding to the entry receptor.

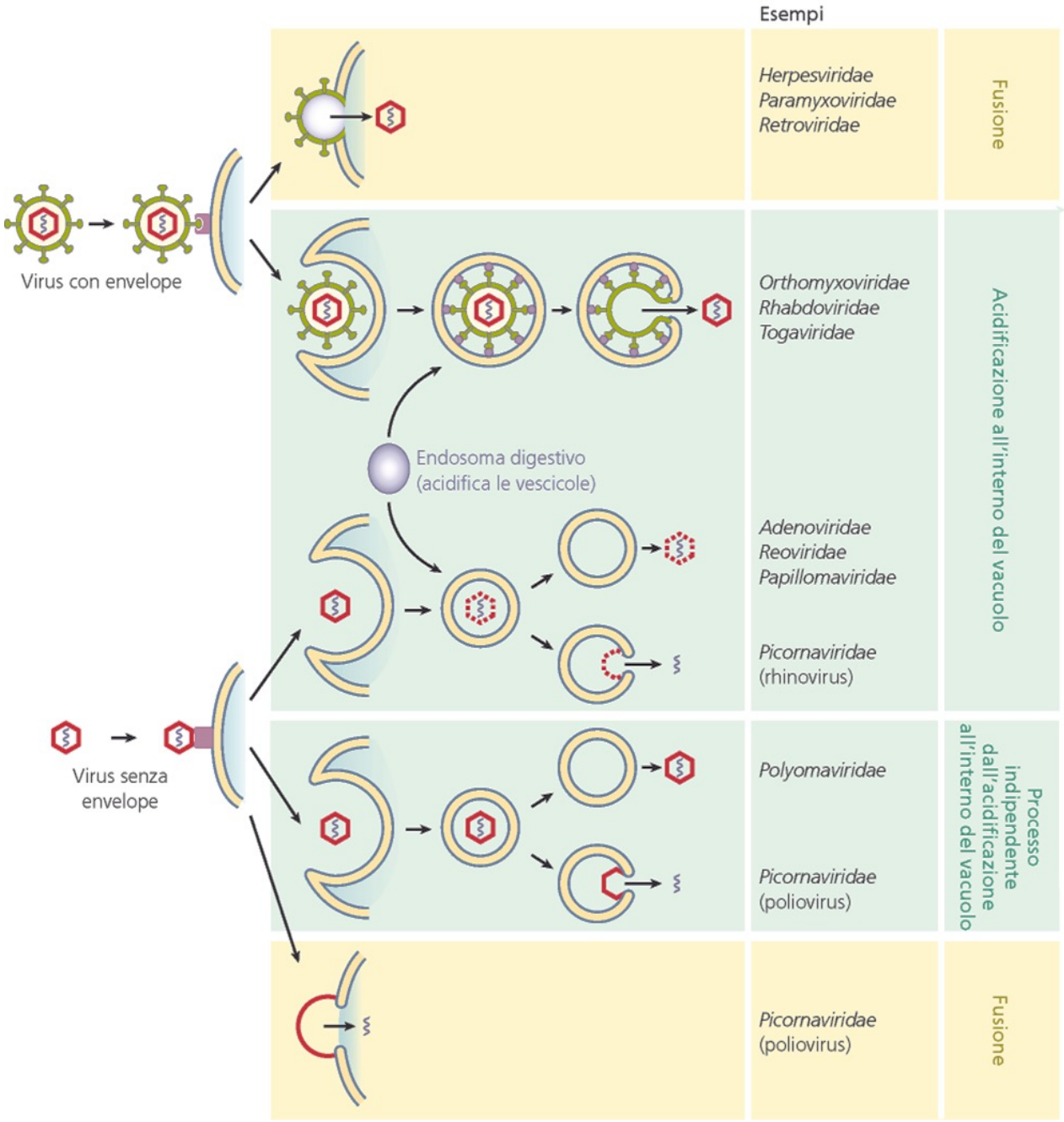
Cell recognition



Some cell attachment factors and receptors for viruses. Schematic diagrams of cell molecules that function during virus entry. GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; LdIr, low-density lipoprotein receptor; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; Car, coxsackievirus-adenovirus receptor.

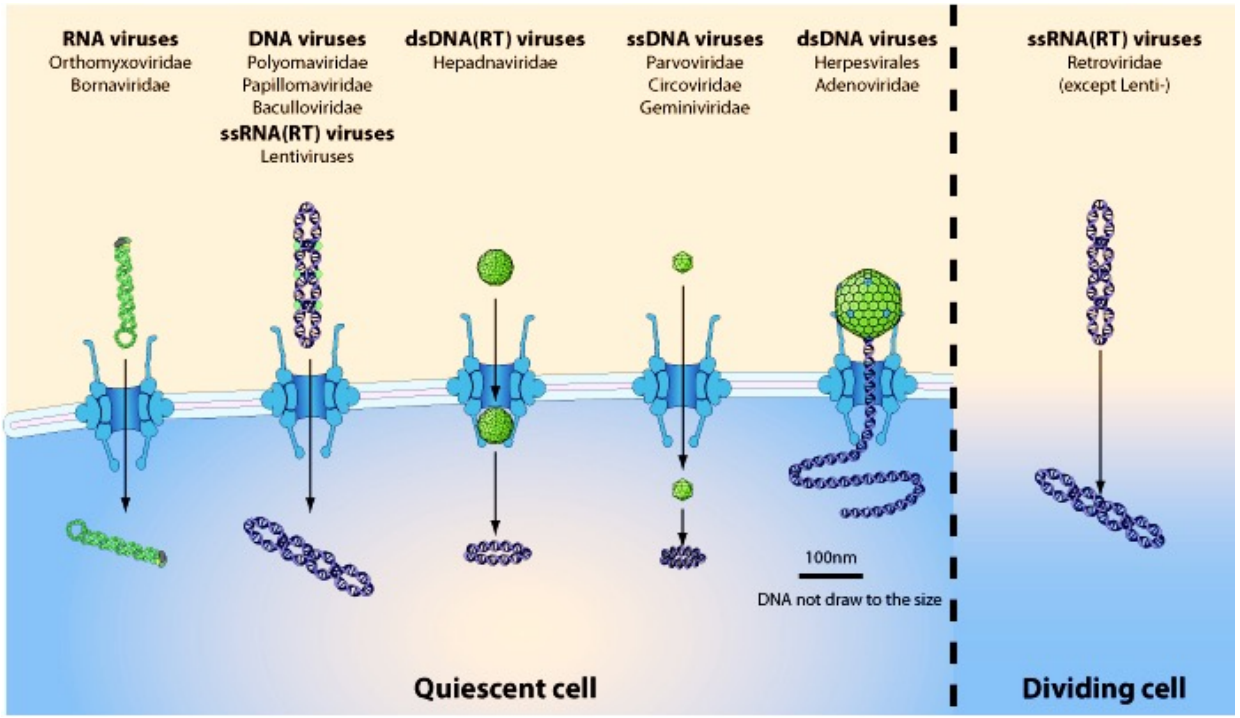
From Flint et al. *Principles of Virology* (2015), ASM Press

Attachment and entry/uncoating



From Harper D., Virus, Zanichelli 2013

Viral penetration into host nucleus



Most DNA and few RNA viruses target their genome to the host nucleus. The crossing of nuclear membrane occurs in several ways:

-RNA virus, dsDNA virus and lentivirus genomes enter via the nuclear pore complex (NPC) through the cellular Importin transport.

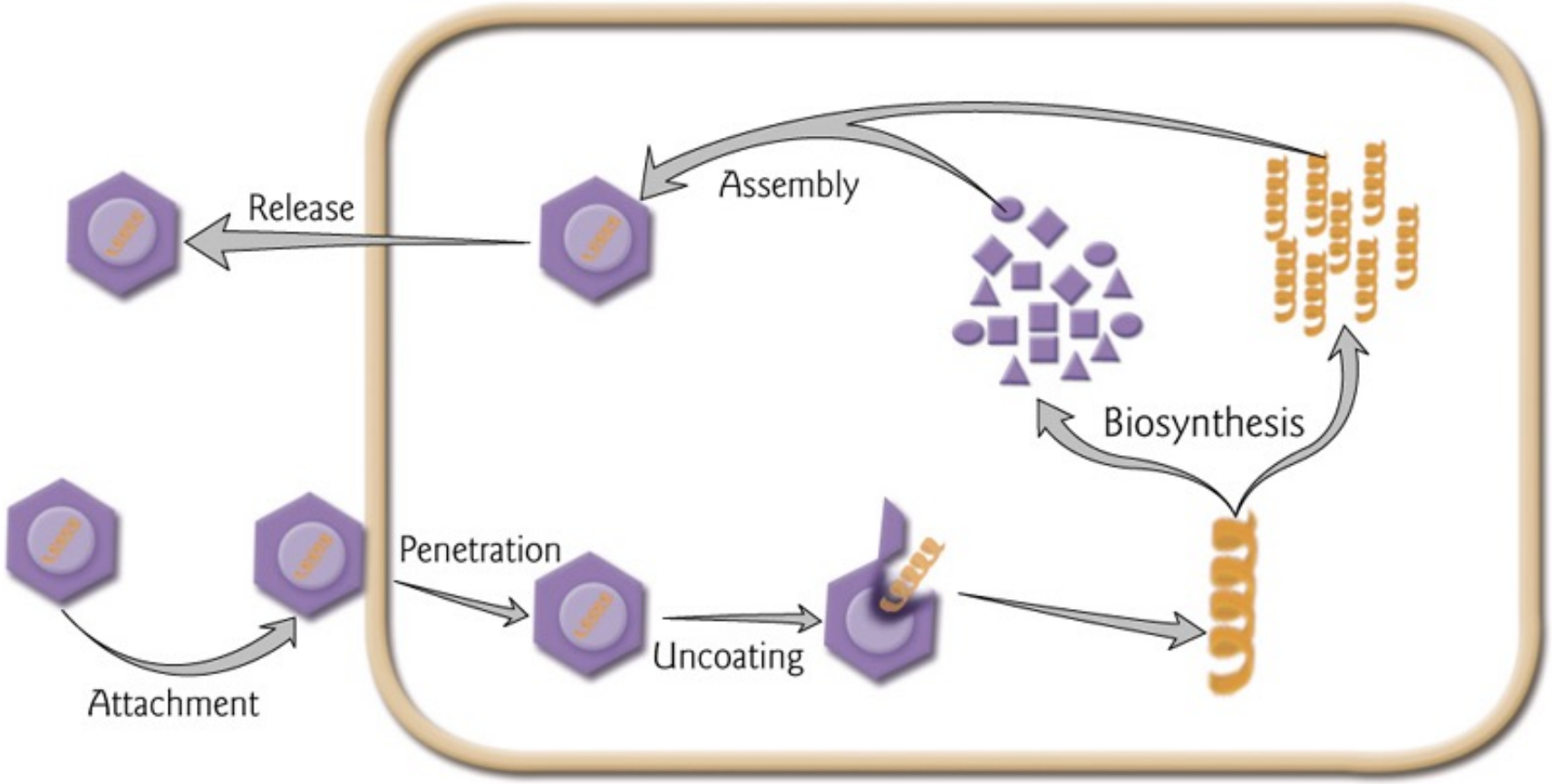
-ssDNA virus capsid seems to be small enough to cross the NPC and enter the nucleus as an intact capsid.

-Hepadnaviridae capsid would enter the NPC pore, but remains attached to it and releases the viral genomic DNA into the nucleoplasm.

-Herpesvirales capsid is too large to enter the NPC pore, the viral genome is directly injected through the NPC on which the capsid docks.

-All retroviridae except lentivirus would enter the nucleus during mitosis, when the nuclear membrane temporarily disintegrates.

Viral genome drives the formation of new viral particles, which are the result of viral component assembly



From Dimmock NJ, Introduction to Modern Virology, Blackwell, 2008

What information is encoded in a viral genome?

Gene products and regulatory signals required for

- replication of the genome
- efficient expression of the genome
- assembly and packaging of the genome
- regulation and timing of the reproduction cycle
- modulation of host defences
- spread to other cells and hosts

Information not contained in viral genomes

• genes encoding a complete protein synthesis machinery (e.g., no ribosomal RNA and no ribosomal or translation proteins);
note: the genomes of some large DNA viruses contain genes for transfer RNAs (tRNAs), aminoacyl-tRNA synthetases, and enzymes that participate in sugar and lipid metabolism

• genes encoding proteins of energy metabolism or membrane biosynthesis

• telomeres (to maintain genomes) or centromeres (to ensure segregation of genomes)

The Baltimore scheme (replication classes)

*Baltimore classification system is based on the central role of translational machinery and places mRNA in the centre. It describes the pathways to form mRNA from DNA or RNA genomes. Viruses can replicate DNA and/or RNA, synthesize RNA from DNA or vice versa, but lack a complete system to make proteins, for which they must rely on host cell ribosomes. Host cells on the other hand can synthesize proteins only from +mRNA strands. Irrespective of the genomic nature of viruses, all viruses must synthesize viral + mRNAs to produce viral proteins "**no exception to date**".*

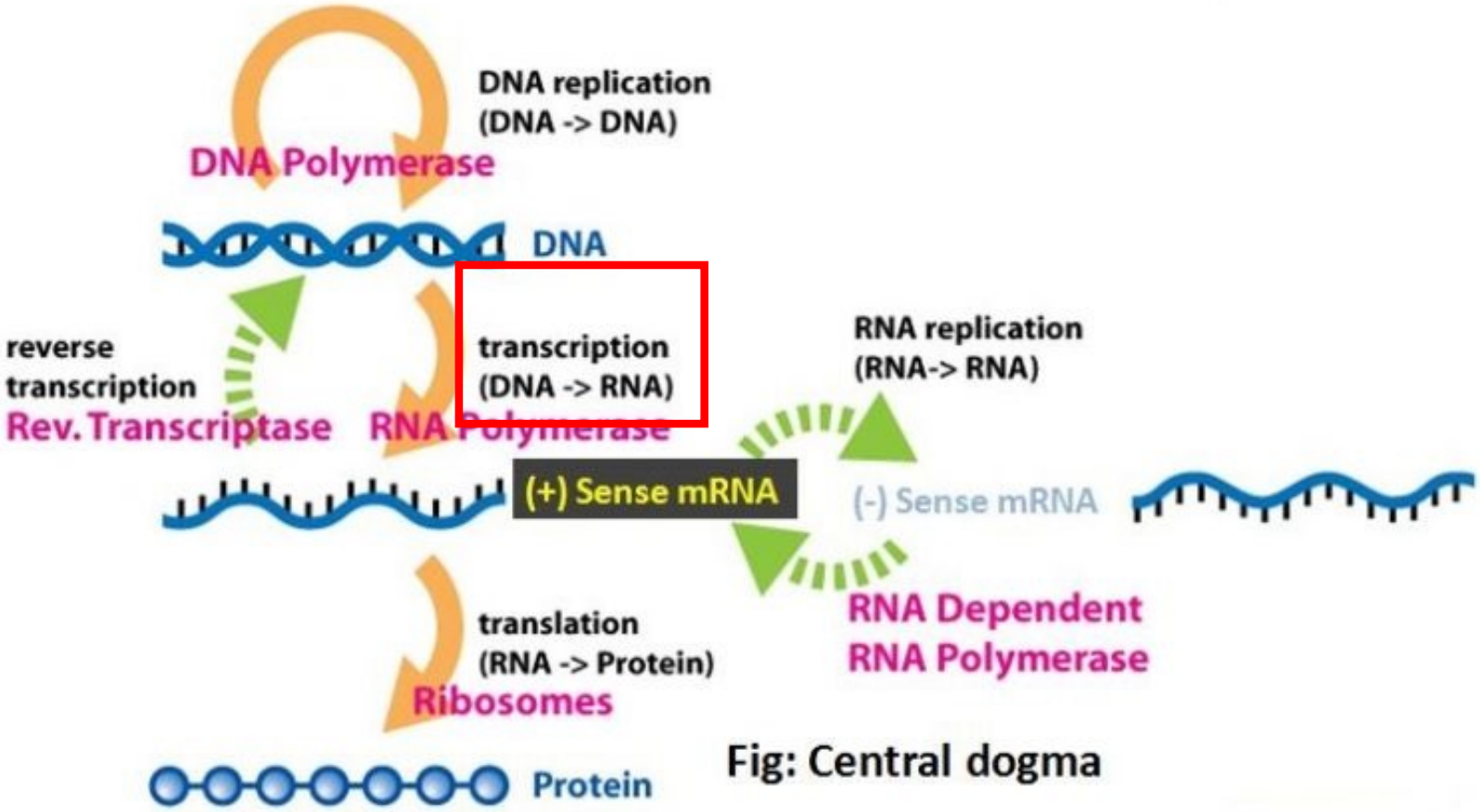


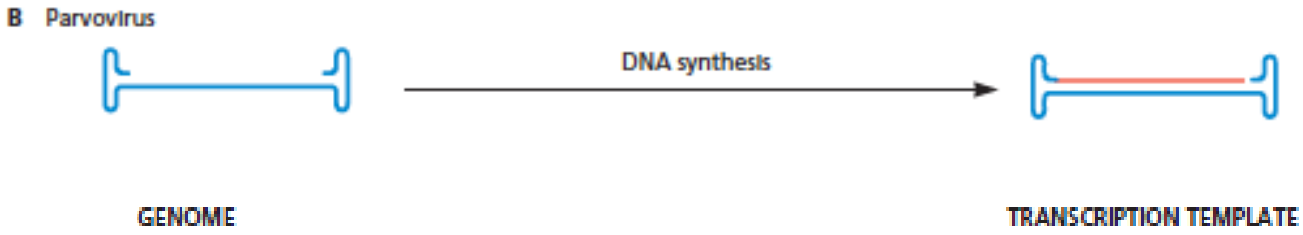
Fig: Central dogma

Transcription of viral genome by host RNApol II

Double-stranded DNA molecules can be transcribed as soon as they reach the nucleus.

Other viral DNA genomes must be converted from the form in which they enter the cell to double-stranded molecules that serve as transcriptional templates. The hepadnaviral genome is an incomplete circular DNA molecule with a large gap in one strand that is repaired by cellular enzymes to form a fully double-stranded DNA molecule.

Similarly, single-stranded genomes such as that of the adenovirus-associated virus, a parvovirus, are converted to double-stranded molecules by a cellular DNA polymerase



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The Baltimore scheme (replication classes)

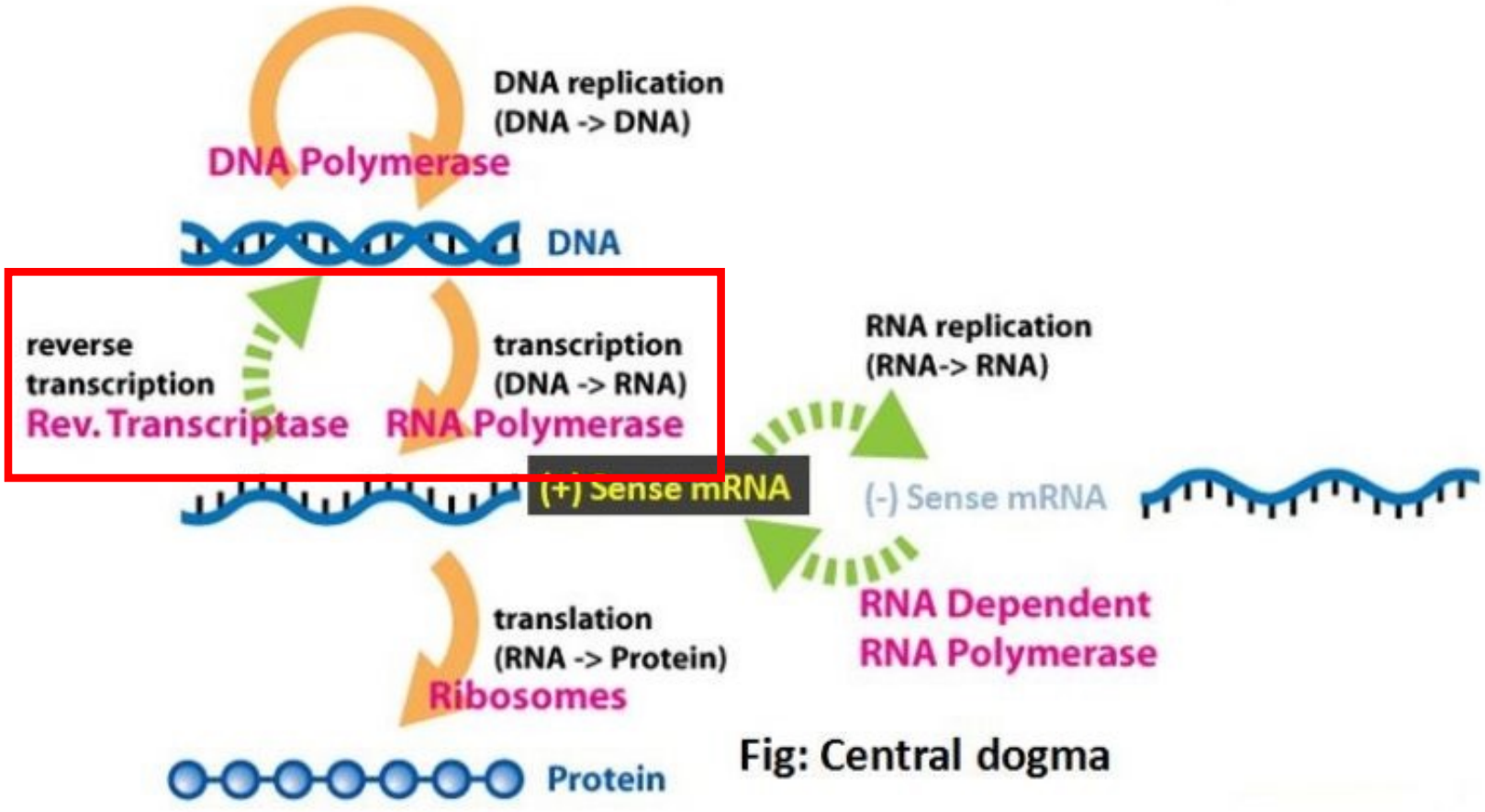
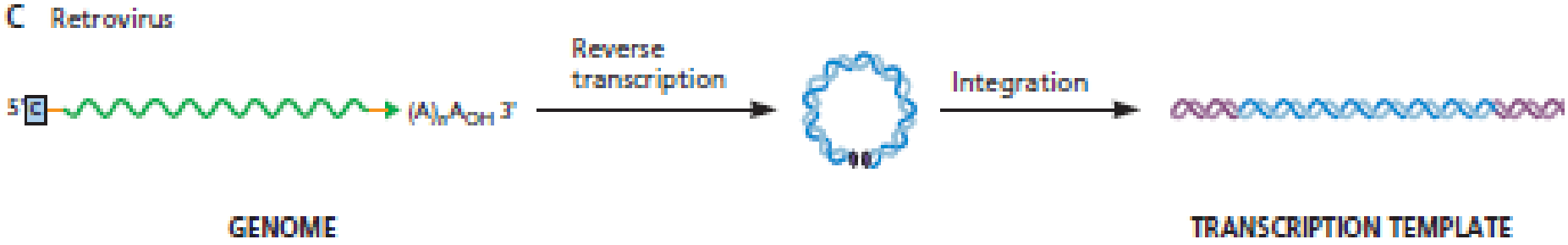


Fig: Central dogma

Transcription of viral genome by host RNApol II

The prerequisites for expression of retroviral genetic information are even more demanding, the (+) strand RNA genome must be both converted into viral DNA and integrated into the cellular genome. Reverse transcription creates an appropriate double-stranded DNA template that includes the signals needed for its recognition by components of the cellular transcriptional machinery



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The Baltimore scheme (replication classes)

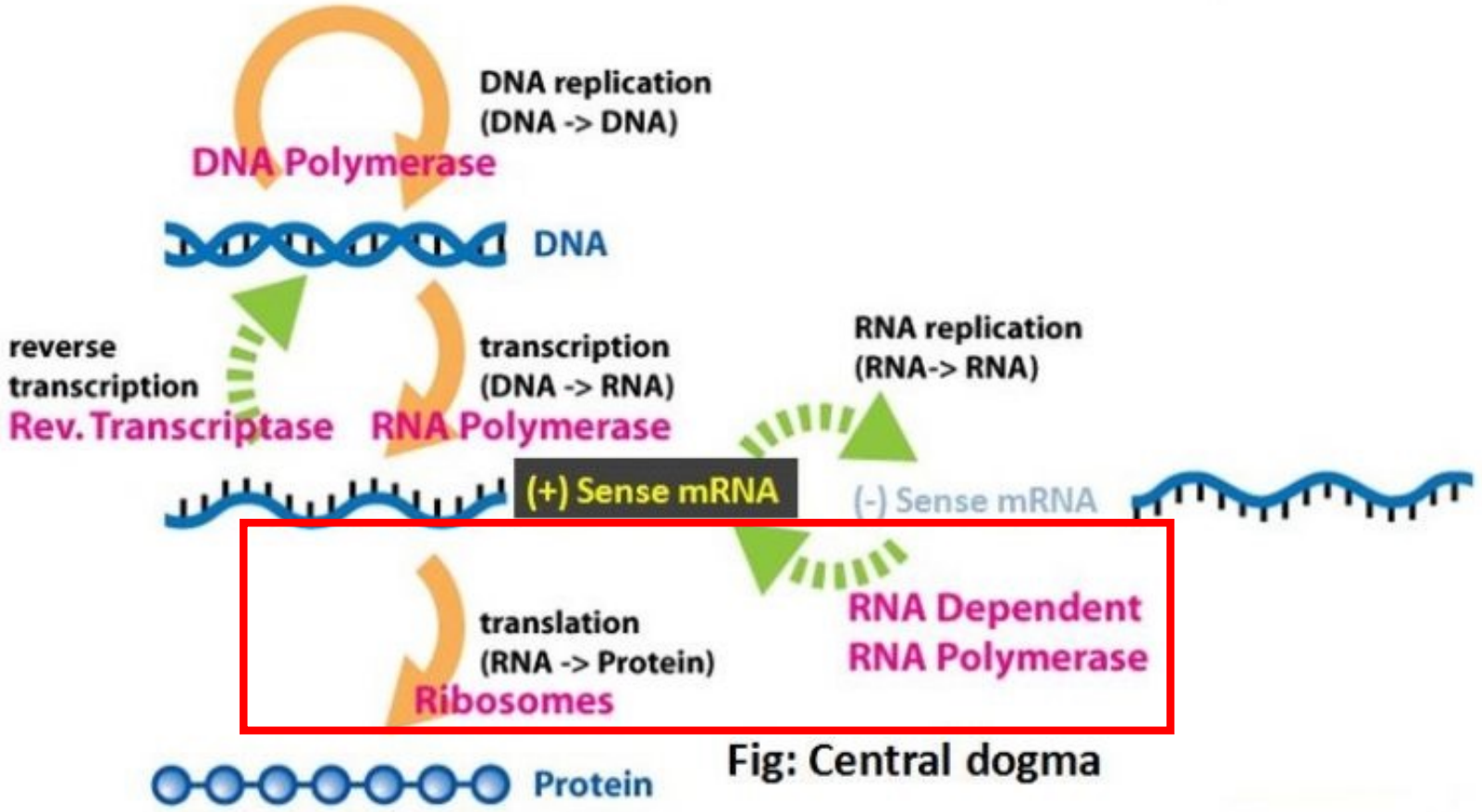
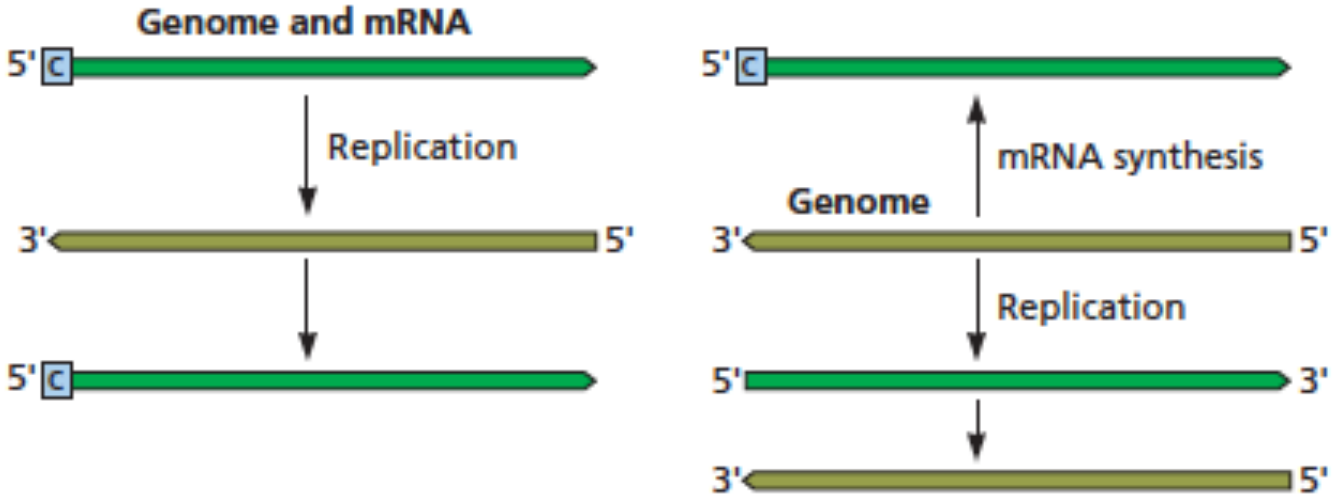


Fig: Central dogma

Synthesis of RNA from RNA Templates (III, IV and V classes)

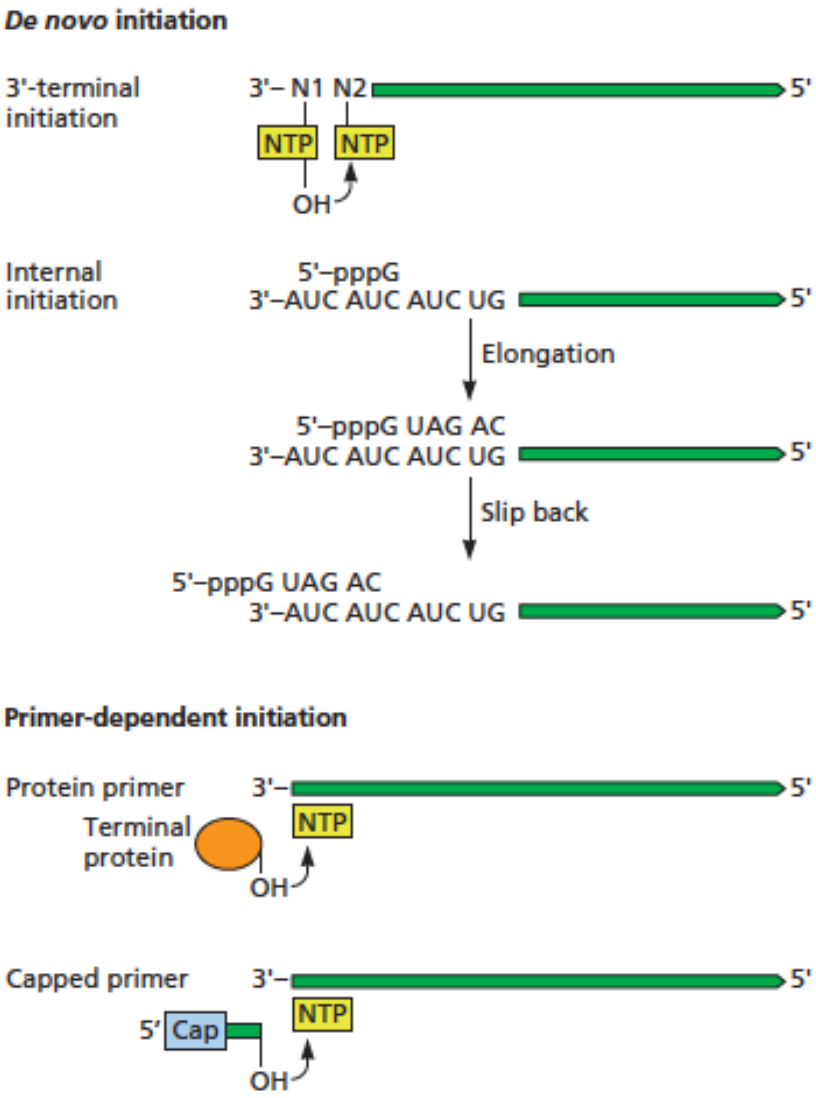
Viral RNA genomes must be copied to provide both mRNAs for the synthesis of viral proteins, and genomes for assembly into progeny virus particles. The synthesis of these RNA molecules is a unique process that has no parallel in the cell. The genomes of all RNA viruses except retroviruses encode an RNA-dependent RNA polymerase to catalyze the synthesis of mRNAs and new genomes.



RNA synthesis by RNA-dependent RNA polymerase is error prone, and this process, together with reassortment and recombination, yields diversity that is required for viral evolution.

From Flint et al. *Principles of Virology* (2015), ASM Press

Synthesis of RNA from RNA Templates (III, IV and V classes)



Mechanisms of initiation of RNA synthesis.

De novo initiation may occur at the 3' end of the viral RNA or from an internal base.

When a primer is required, it may be a capped or protein-linked oligonucleotide.

From Flint et al. *Principles of Virology* (2015), ASM Press

Review
Cancer Sci. 2020 Nov;111(11):3976-3984.
doi: 10.1111/cas.14618. Epub 2020 Sep 12.

RNA-dependent RNA polymerase, RdRP, a promising therapeutic target for cancer and potentially COVID-19

Mitsuhiro Machitani[1], Mami Yasukawa[1], Jotaro Nakashima[1], Yasuhiro Furuichi[2], Kenkichi Masutomi[1]

Abstract

A recent outbreak of coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 has driven a global pandemic with catastrophic consequences. The rapid development of promising therapeutic strategies against COVID-19 is keenly anticipated. Family Coronaviridae comprises positive, single-stranded RNA viruses that use RNA-dependent RNA polymerase (RdRP) for viral replication and transcription. As the RdRP of viruses in this family and others plays a pivotal role in infection, it is a promising therapeutic target for developing antiviral agents against them. A critical genetic driver for many cancers is the catalytic subunit of telomerase: human telomerase reverse transcriptase (hTERT), identified initially as an RNA-dependent DNA polymerase. However, even though hTERT is a DNA polymerase, it has phylogenetic and structural similarities to viral RdRPs. Researchers worldwide, including the authors of this review, are engaged in developing therapeutic strategies targeting hTERT. We have published a series of papers reporting that hTERT has RdRP activity and that this RdRP activity in hTERT is essential for tumor formation. Here, we review the enzymatic function of RdRP in virus proliferation and tumor development, reminding us of how the study of the novel coronavirus has brought us to the unexpected intersection of cancer research and RNA virus research.