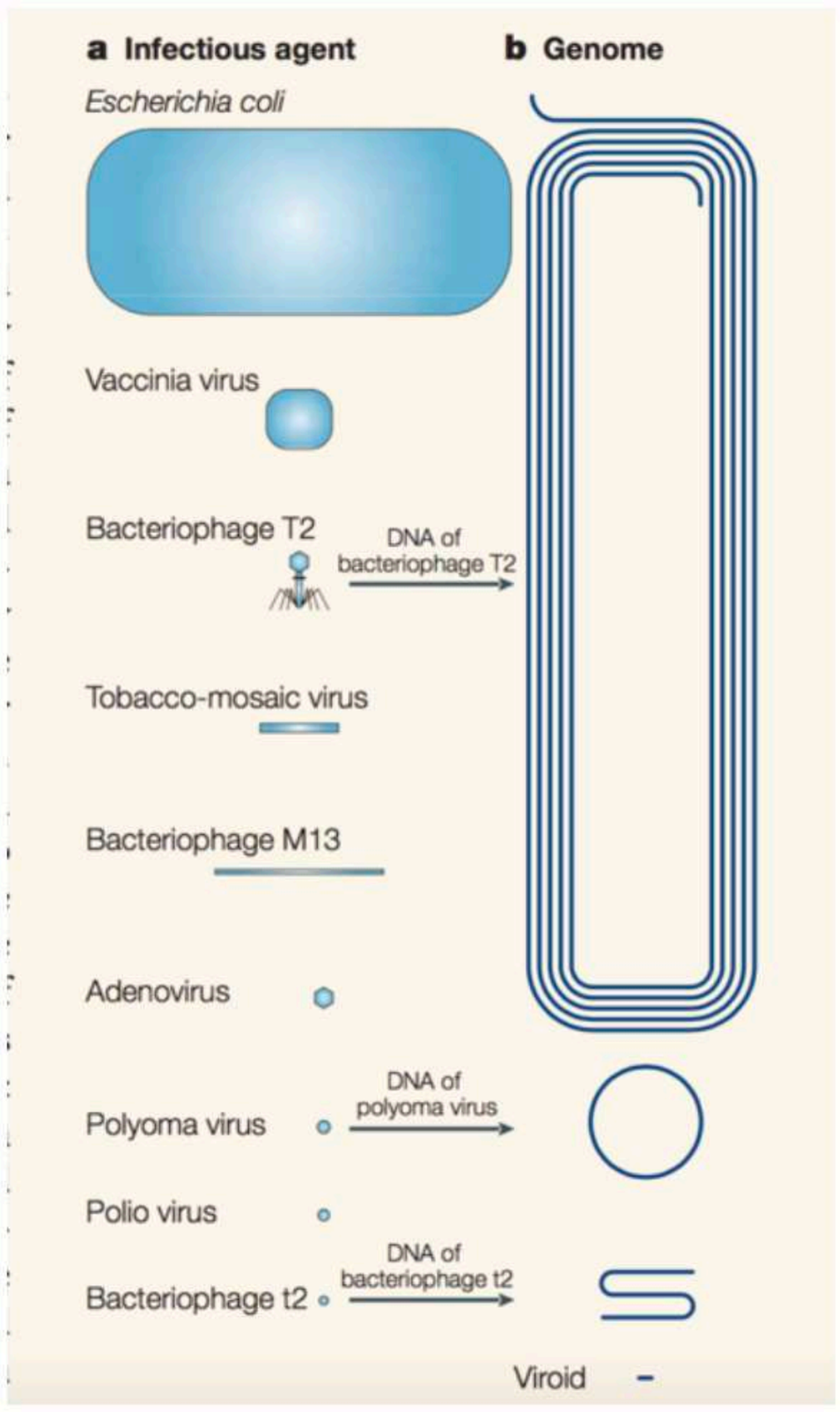
L04a

Recap

Che domande avete per i virus?

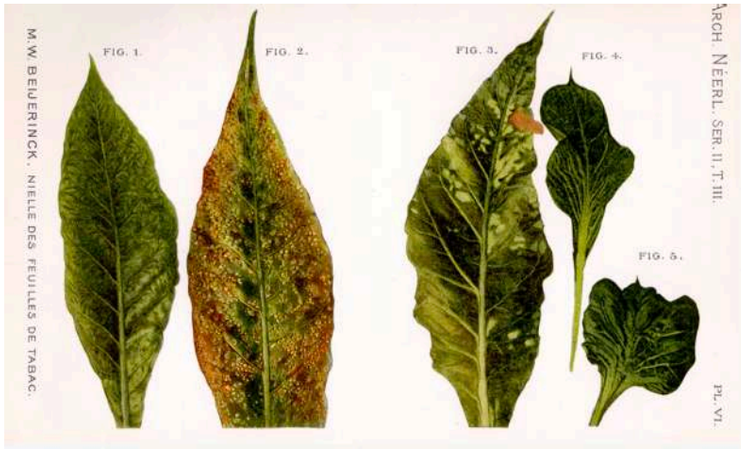
Viruses…. scaling down!



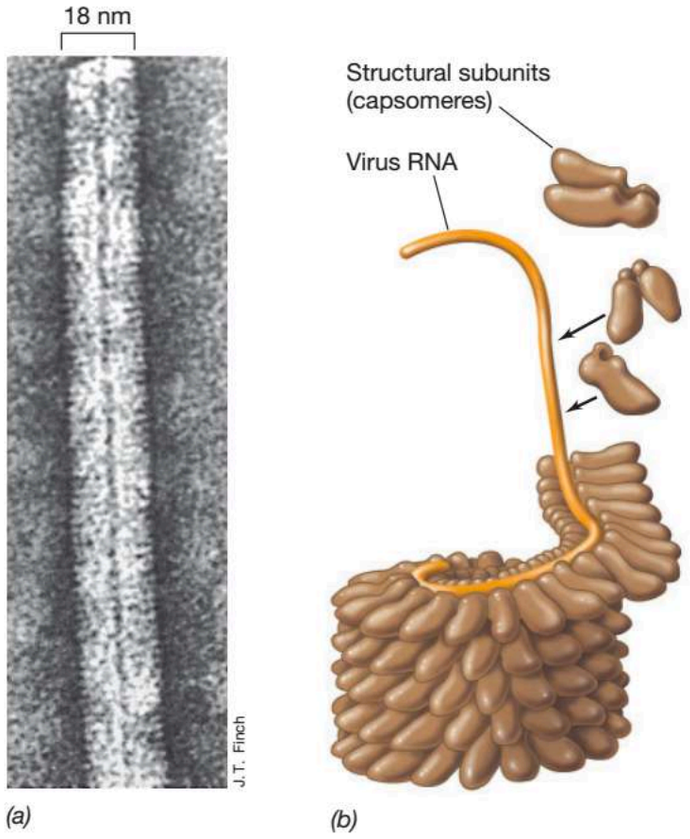
American Scientit, 1983

Viruses

Viruses have streamlined adaptive strategies to replicate themselves



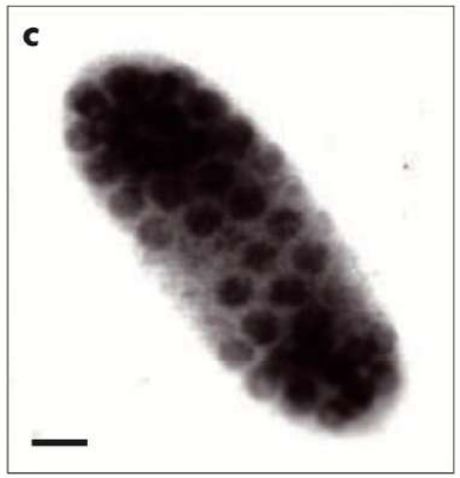
- Beijerinck studied the tobacco “mosaic disease”
- Beijerinck showed that the infectious agent in this disease **< bacterium** and that it somehow became incorporated into cells of the living host plant: "**<u>contagium vivum fluidum</u>**"
- Beijerinck described not only the first virus but also the basic principles of virology
- Ivanovski in 1892 is considered father of virology, continued on tobacco “mosaic disease”



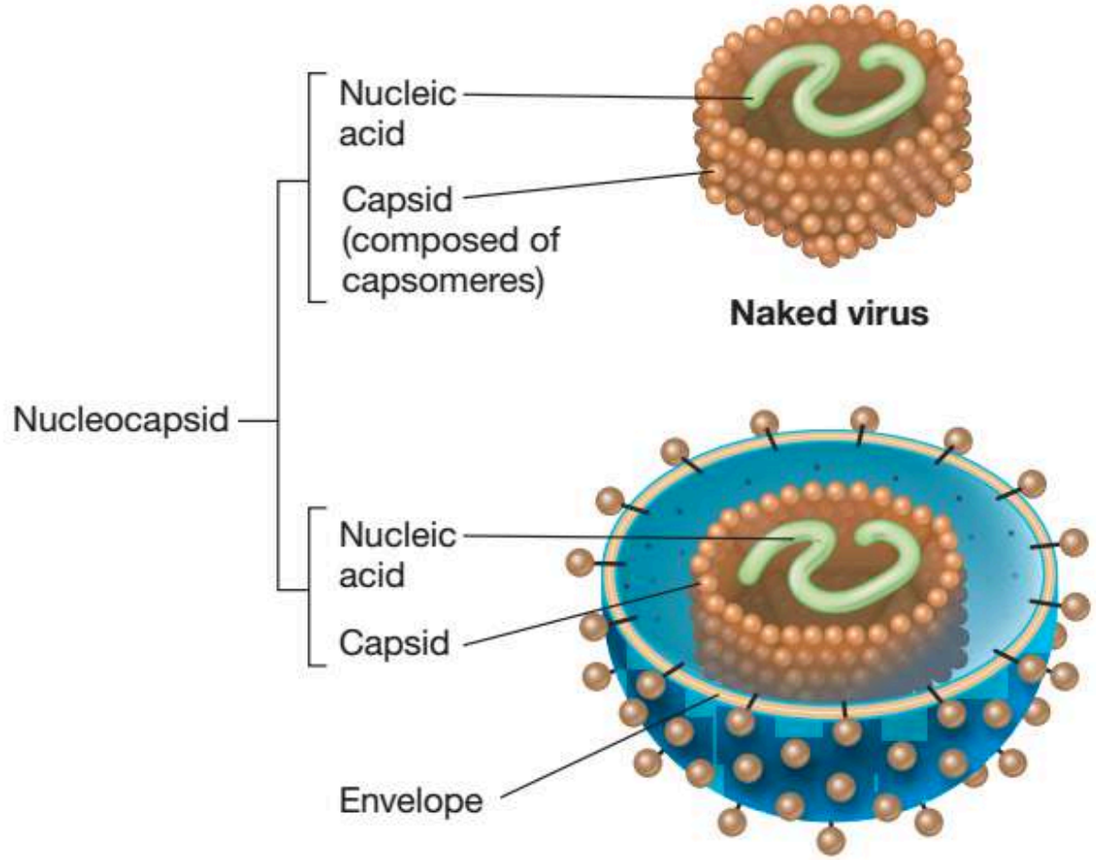
Madigan et al. 2020

https://www.tudelft.nl/en/scd/collectie-tentoonstelling/delft-school-of-microbiology/delft-school-of-microbiology/founding-fathers/martinus-willem-beijerinck/the-professor/viruses/

Virus: part I



Weinbauer and Willhelm

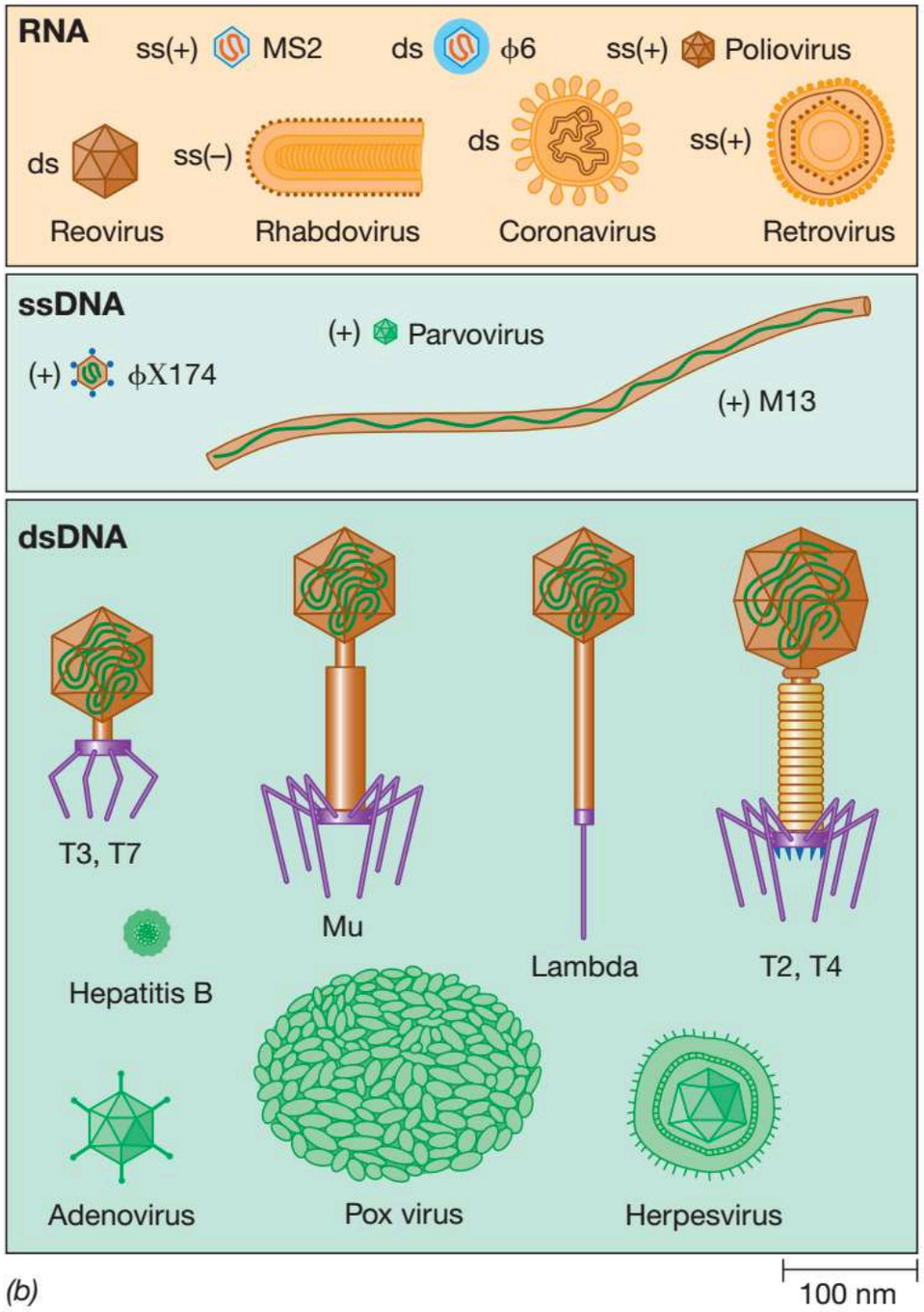


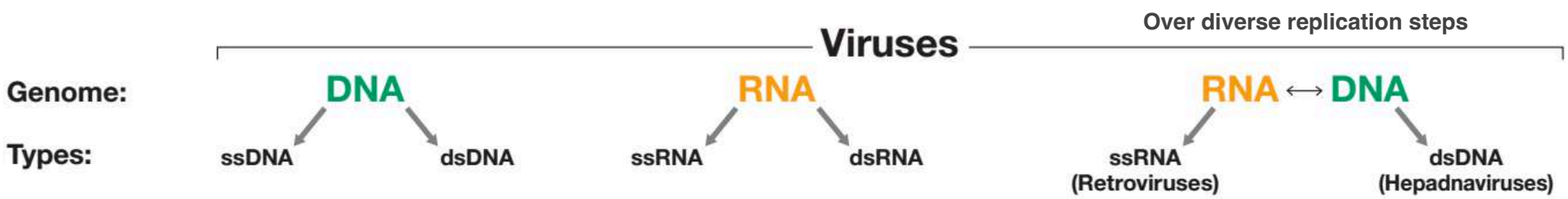
Madigan et al. 2020

- Not found on the tree of life (based on 16SrRNA gene)
- Not truly alive,
- Are obligate parasites (replication within the host cell cytoplasm of a host cell)
- Are not cells (no cytoplasmic membrane, cytoplasm & ribosomes)
- Cannot conserve energy
- Do not carry out metabolic processes
- Naked vs Enveloped virus (phospholipid bilayer taken from the host cell membrane) and viral proteins

Virus: part II

- Take over the metabolic systems of infected cells
- Genomes composed of DNA or RNA that can be either double- or single-stranded
- Viral genomes are often quite small
- No genes are conserved among all viruses, or between all viruses and all cells

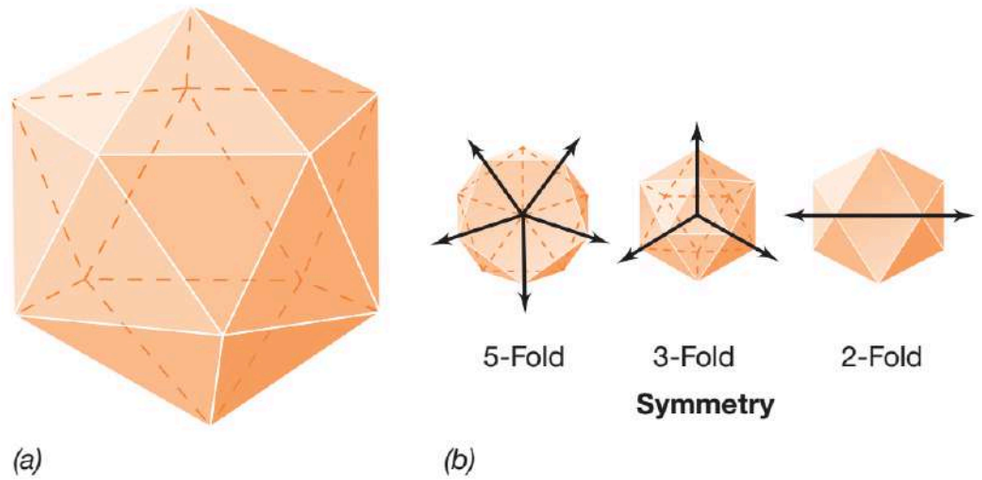


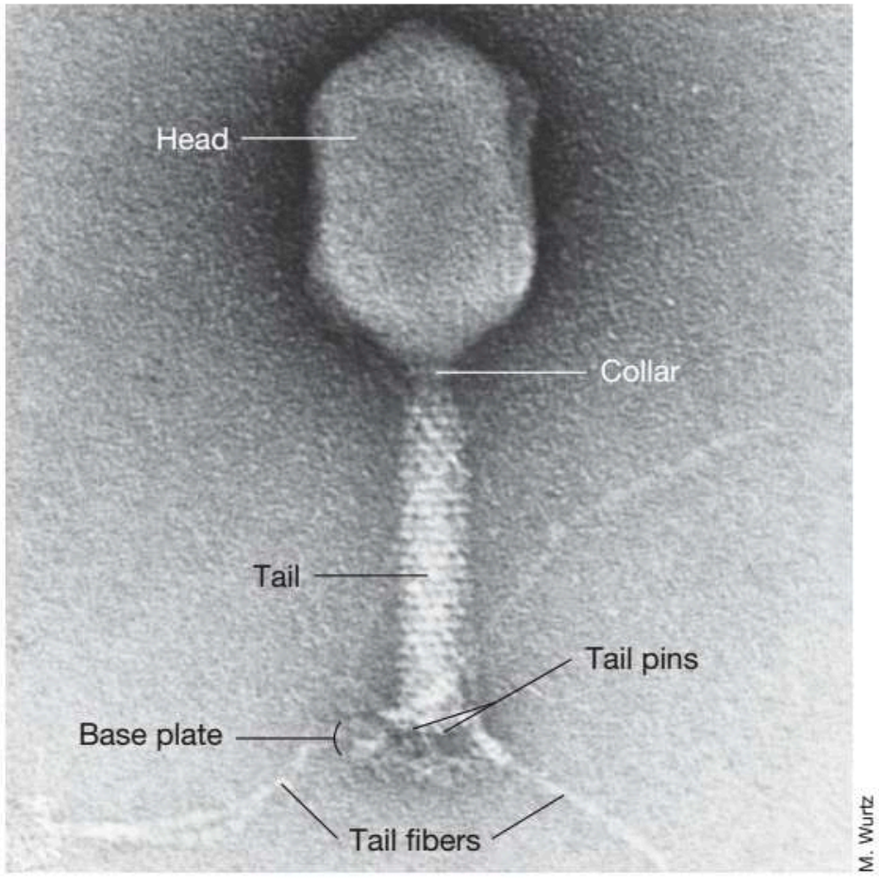


Madigan et al. 2020

Virus: part III

- Very symmetrical
- Rod-shaped viruses have helical symmetry
- Spherical viruses have icosahedral symmetry
- Head-plus tail bacteriophages of *E. coli*





- Virion is the extracellular form of the virus
- Virus —> Bacteria & Archaea only nucleic acid enters the cell
- Virus —> Eukarya entire virion is taken up
- Diverse evolution in infection pathways

Madigan et al. 2020

Viruses *vs* Cells

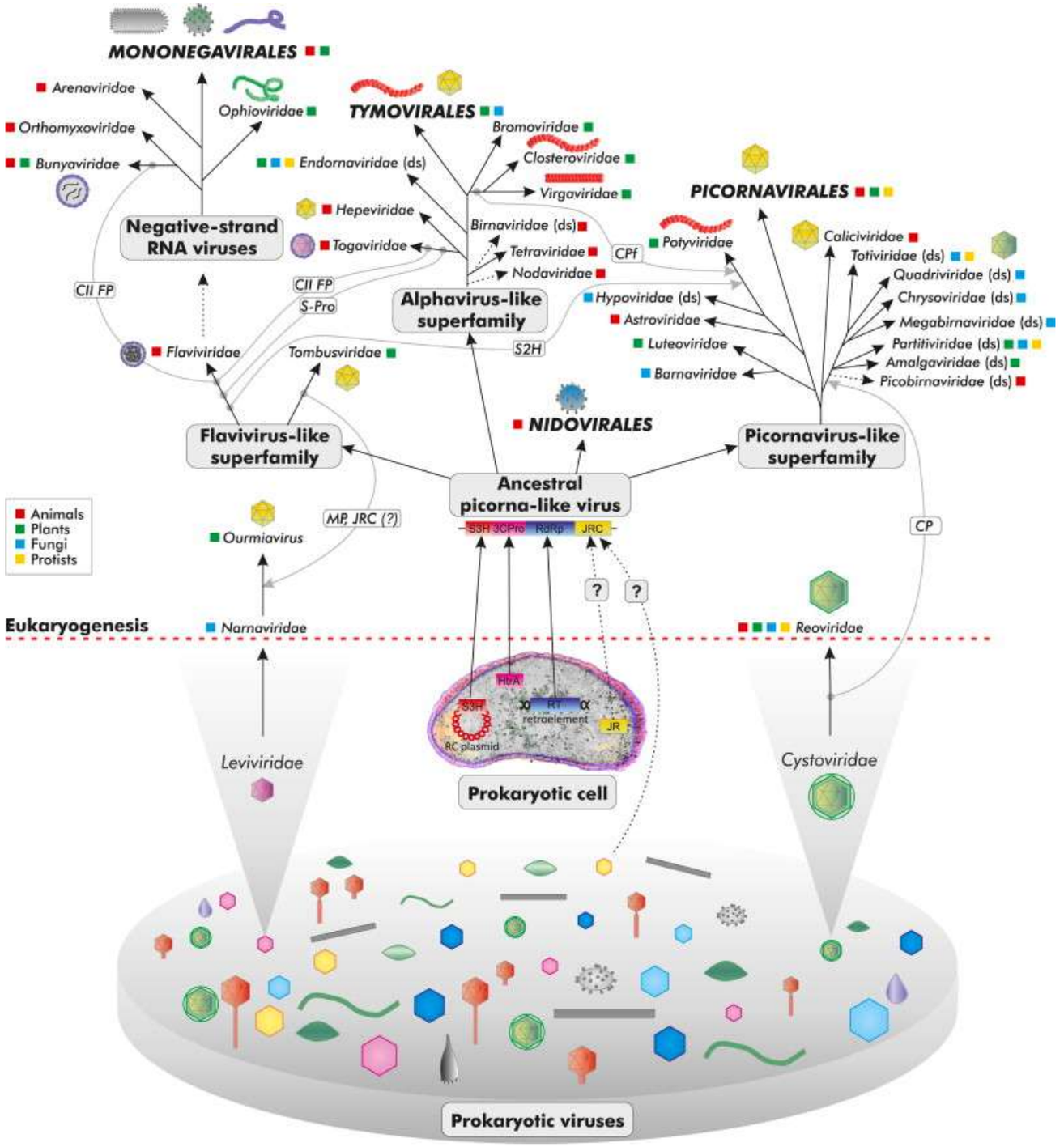
Table 1 | **Comparison of cellular and viral traits**

Trait	Cells	Viruses
Information content	Yes	Yes
Self-maintenance	Yes	No
Self-replication	Yes	No
Evolution	Yes	By cells
Common ancestry	Yes	No
Structural historical continuity	Yes	No
Genes involved in carbon metabolism	Yes	Cellular origin
Genes involved in energy metabolism	Yes	Cellular origin
Genes involved in protein synthesis	Yes	Cellular origin

Moreira & López-García 2009

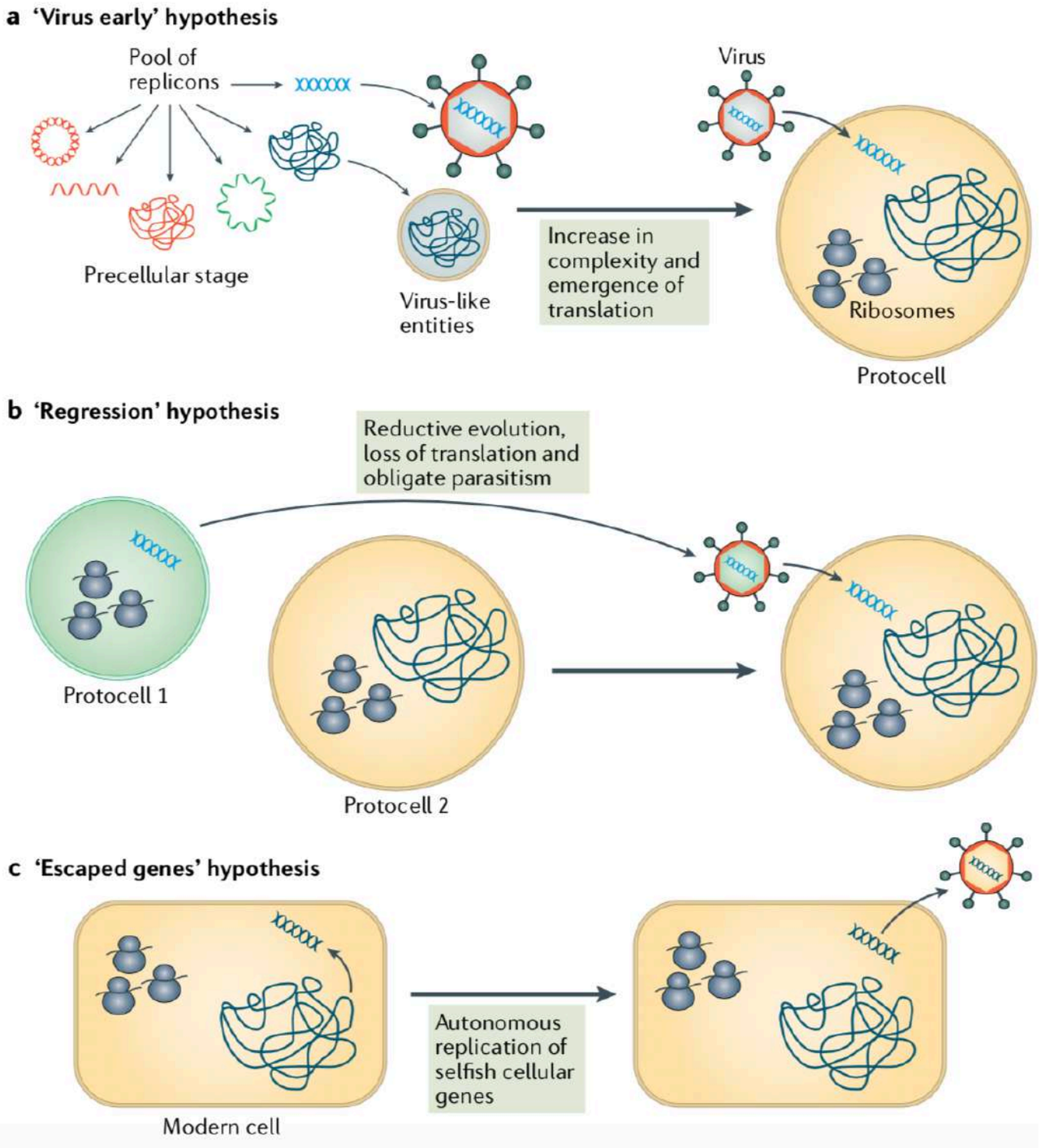
Viruses are critically important microbes whose replication shows parallels with the growth of microbial cells that are under natural selection

Origin of the major groups of RNA viruses of eukaryotes



Koonin et al. 2015

Origin of viruses - viruses are relic from RNA world



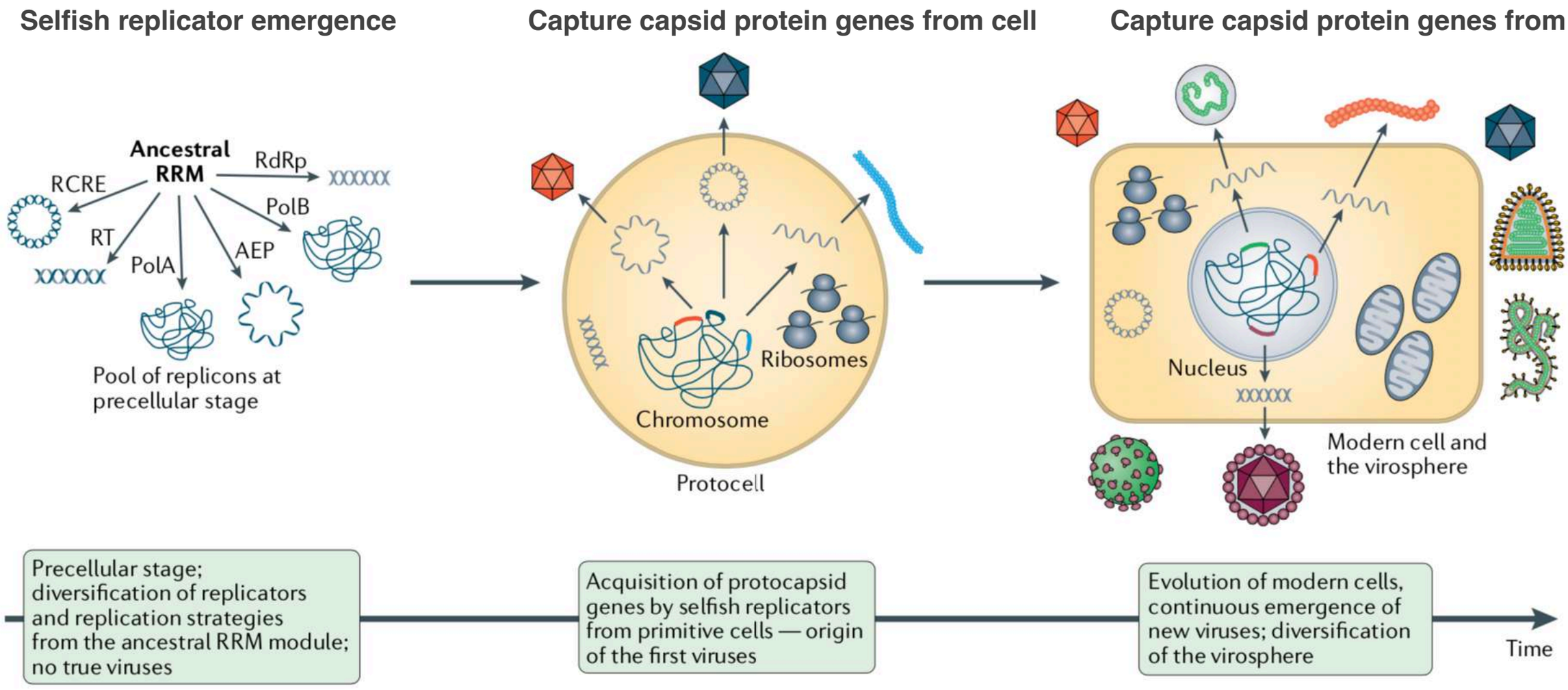
Kuprovic et al., 2019

- From early replicative elements prior LUCA
- During RNA world

- Emergence from degeneration of cells
- Assumption parasitic lifestyle

- Cellular genes acquire selfish replication and spread

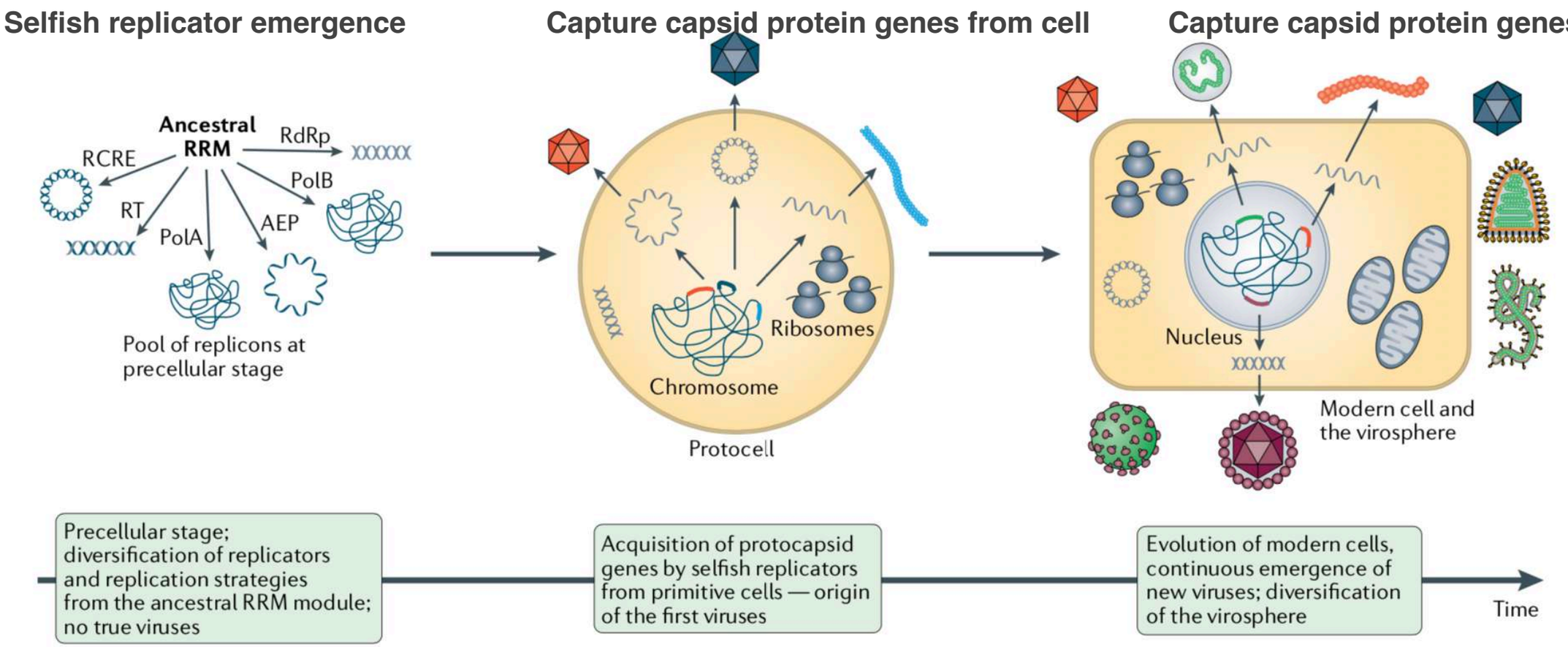
Origin of viruses



Kuprovic et al., 2019

- The **RNA recognition motif (RRM)** is one of the most **common RNA-binding domains** and occurs in all forms of cellular life for RNA biogenesis processes
- **RRM** was one of the **earliest protein domains to evolve** and was central to the origin and early evolution of both **RNA and DNA replication**

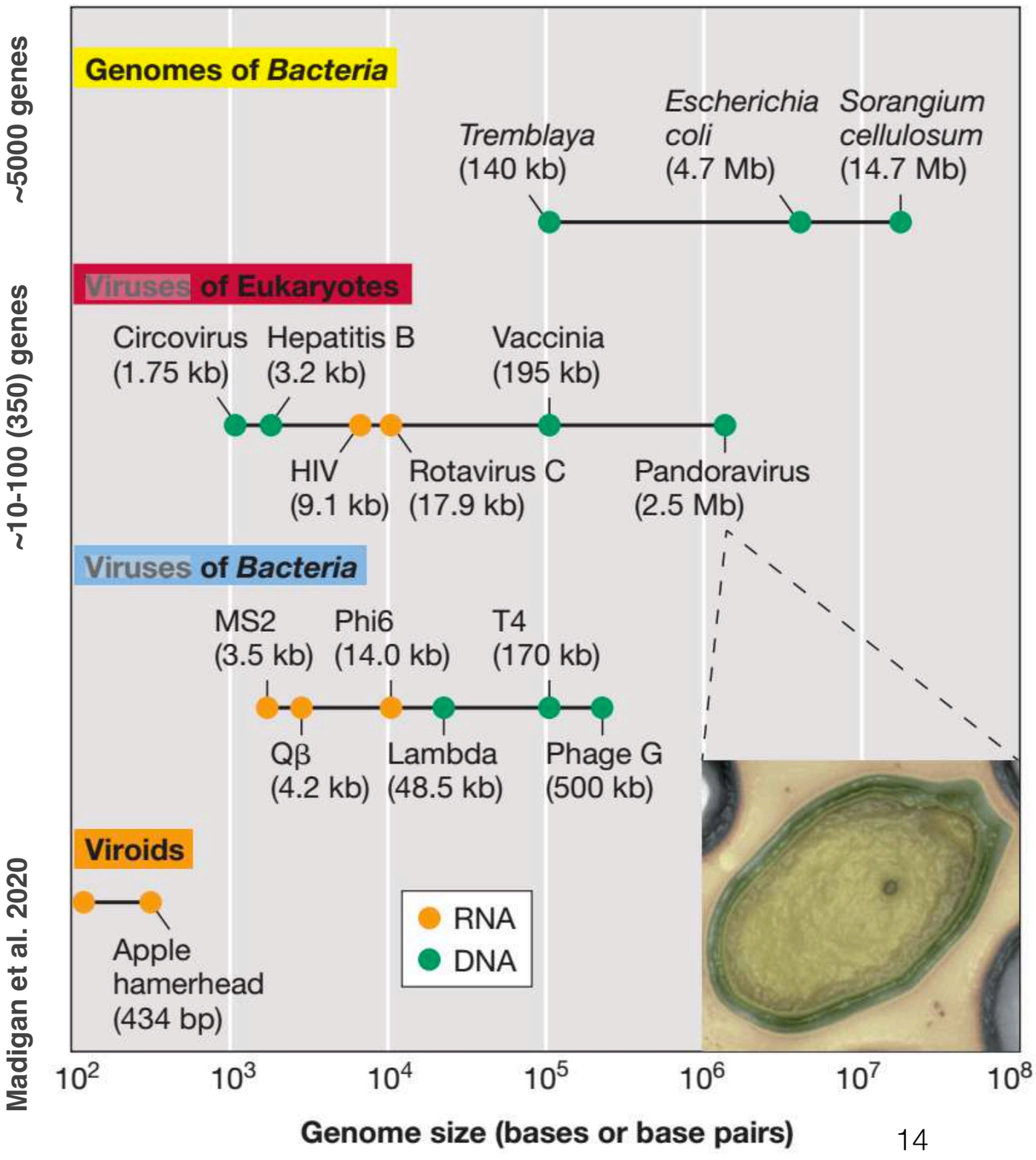
Origin of viruses



Kuprovic et al., 2019

- Structurally **related domains** are widespread in many viruses and **mobile genetic elements**
- Replication enzymes containing **RRM** include:
    - Reverse transcriptase (RT),
    - RNA-dependent RNA polymerase (RdRp),
    - Rolling-circle replication endonuclease (RCRE),
    - DNA-dependent DNA polymerases of families A and B (PolA and PolB)
    - Archaeo-eukaryotic primase (AEP)

Viral and Bacterial genome size range



1 kb = 10^3 bp (base pairs)
1 Mb = 10^6 bp
1 Gb = 10^9 bp

1 kb = 10^{-6} pg
1 Mb = 10^{-3} pg
1 Gb = 1pg

Doležel et al., 2003
Base pair # = mass in pg x 0.978 10^9

1 kb = 0.33 µm
1 Mb = 0.33 mm
1 Gb = 0.33 m

Dickerson et al., 1982
1 bp = 0.33 nm

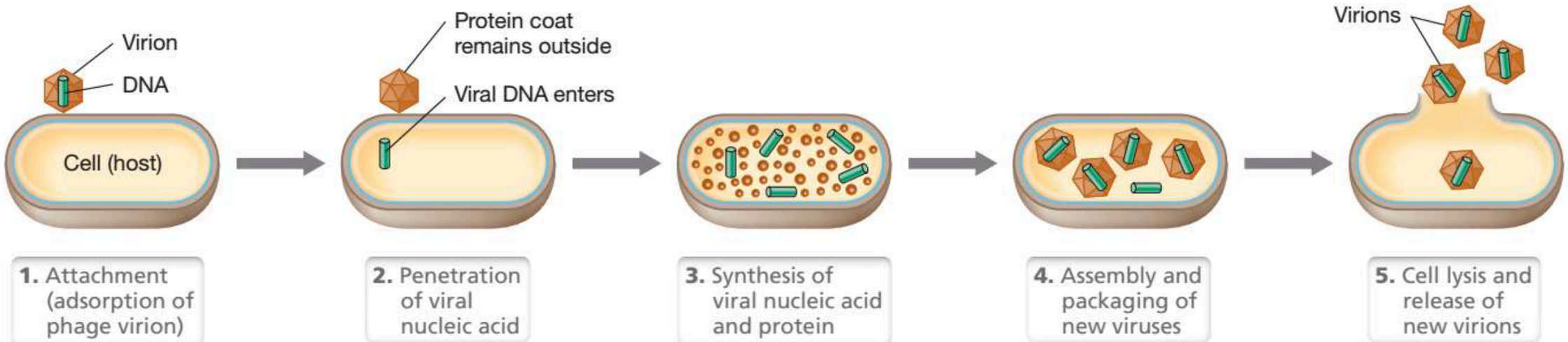
TABLE 8.1 Some bacteriophages of *Escherichia coli*

Bacteriophage	*Virion structure*	*Genome composition*[a]	*Genome structure*	*Size of genome*[b]
MS2	Icosahedral	ssRNA	Linear	3,600
ϕX174	Icosahedral	ssDNA	Circular	5,400
M13, f1, and fd	Filamentous	ssDNA	Circular	6,400
Lambda	Head & tail	dsDNA	Linear	48,500
T7 and T3	Head & tail	dsDNA	Linear	40,000
T4	Head & tail	dsDNA	Linear	169,000
Mu	Head & tail	dsDNA	Linear	39,000

[a]ss, single-stranded; ds, double-stranded.

Madigan et al. 2020

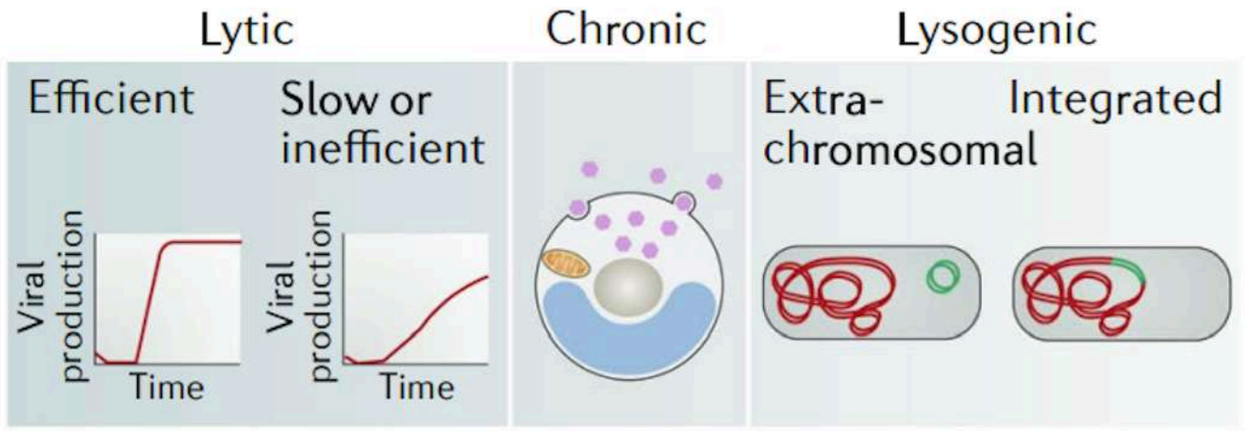
Viral cycle: replication
(Bacteria & Archaea)



Madigan et al. 2020

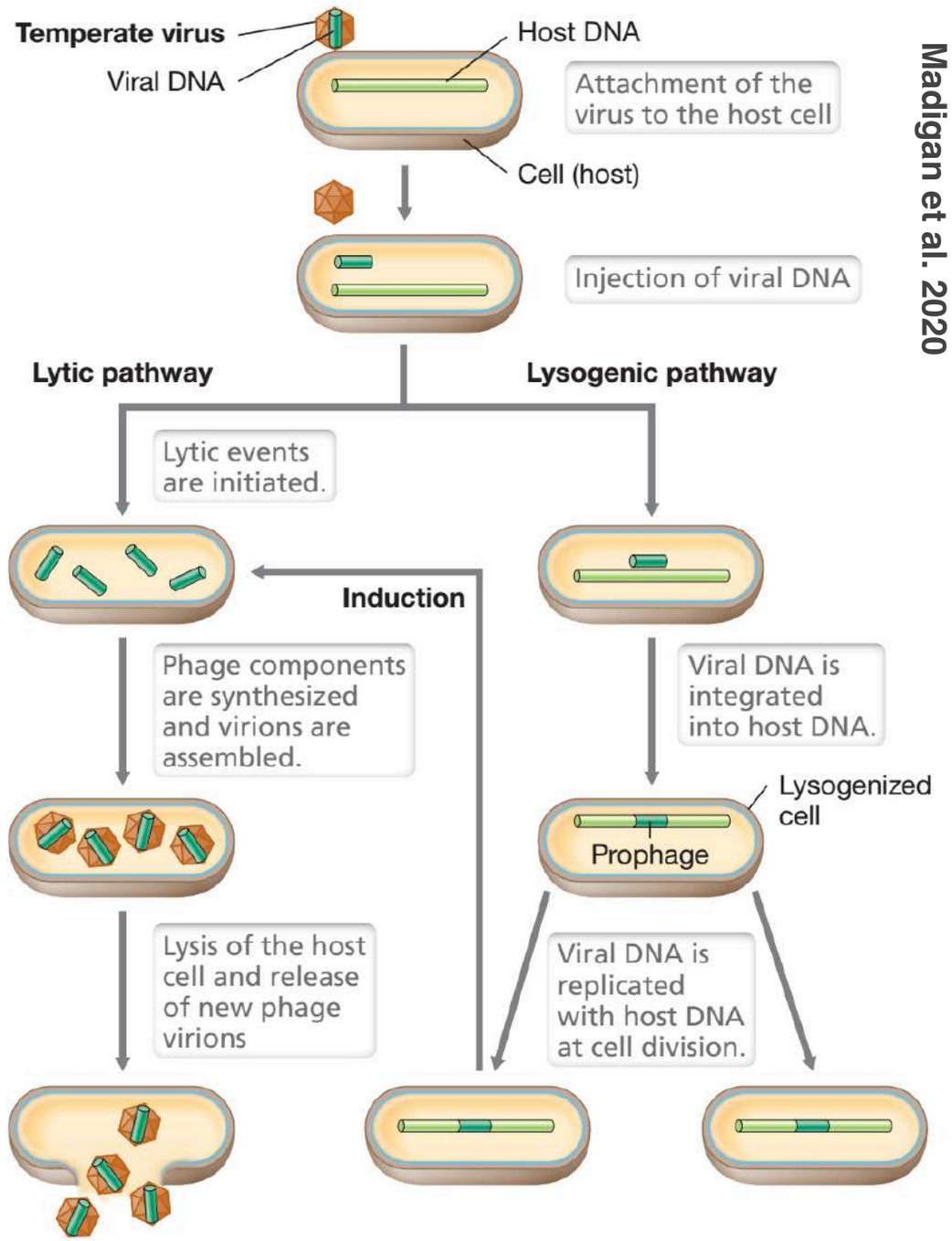
1. **Attachment (adsorption) of the virion to the host cell**
2. **Penetration (entry, injection) of the virion nucleic acid into the host cell**
3. **Synthesis of virus nucleic acid and protein by host cell machinery as redirected by the virus**
4. **Assembly of capsids and packaging of viral genomes into new virions**
5. **Release of new virions from the cell**

Viral life strategies



Zimmermann et al., 2020

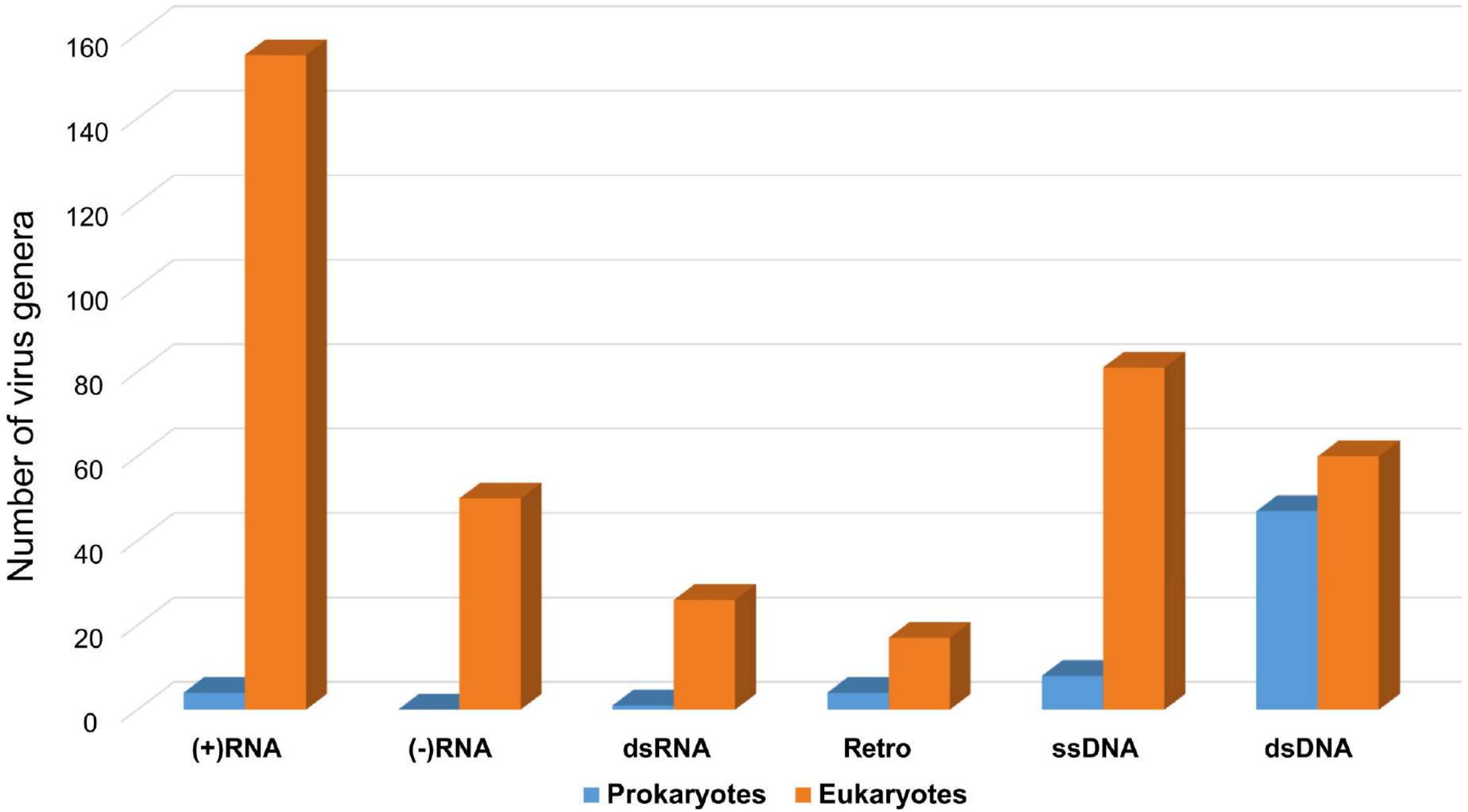
- Viruses are co-evolving with host cells
- 3 major strategies
    1. Lytic —> host death
    2. Chronic, constant shedding visions
    3. Lysogenic, co-existence with host
- Lysogenic cycle depends on the genotype of phage and host, the physiological status of the cell, and phage concentration



Madigan et al. 2020

Burst size

“Baltimore classes” of viruses in prokaryotes and eukaryotes



Koonin et al. 2015

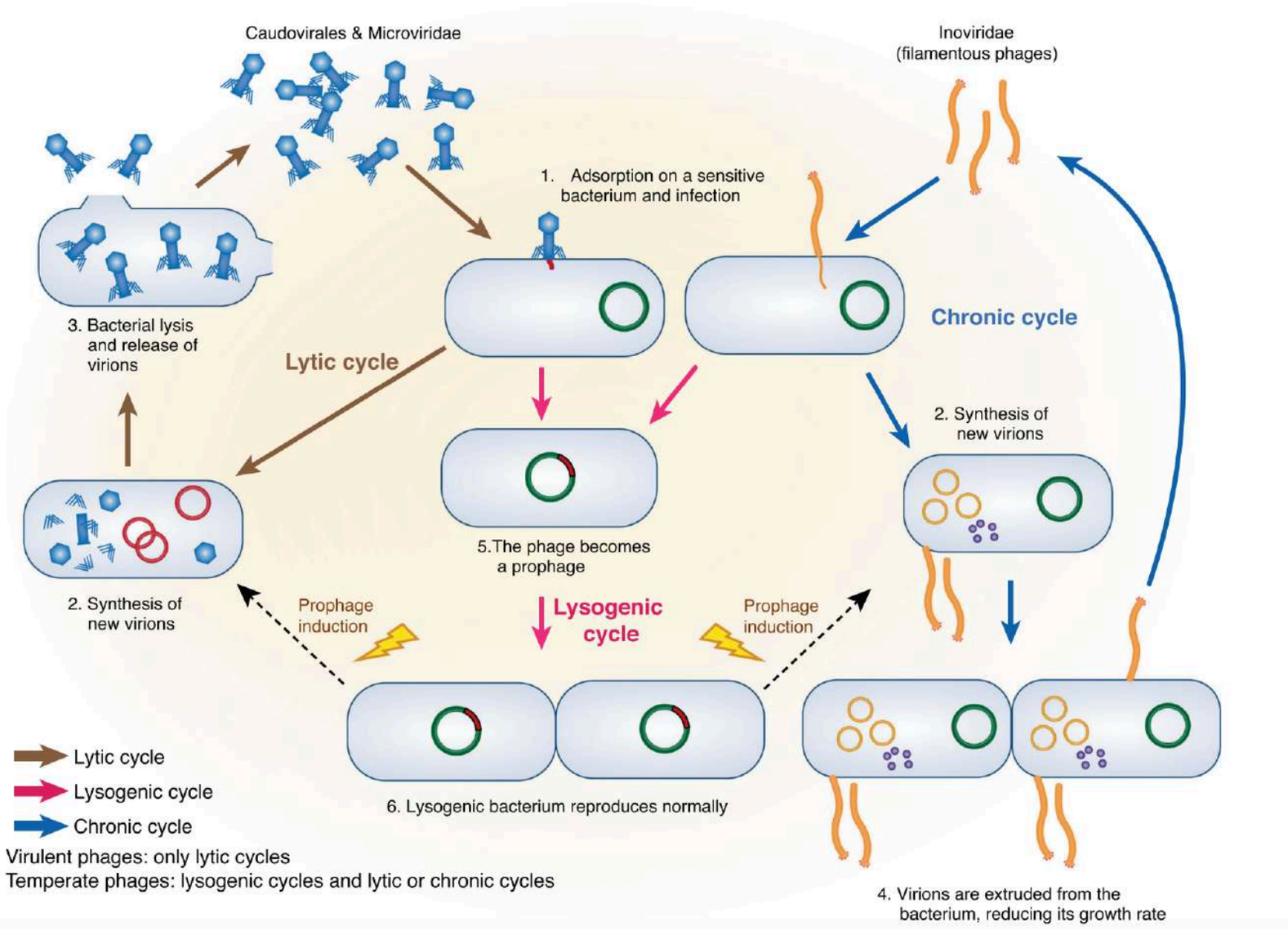
Prey-selection

In both bacteria and archaea, the vast majority of the viruses possess dsDNA genomes, mostly within the range of 10 to 100 kb. The second most common class includes small ssDNA viruses. Positive-strand RNA and dsRNA viruses are extremely rare, and no retroviruses are known (reverse-transcribing elements exist but are not highly abundant)

Eukaryotes host numerous, highly diverse RNA viruses (particularly of the positive-strand class) as well as reverse-transcribing elements and retroviruses that typically integrate into the host genome and are extremely abundant, comprising a substantial fraction of the genome in many groups of eukaryotes

Koonin et al. 2015

In the light of evolution: Intestinal human virioshpere



Sausset et al., 2020

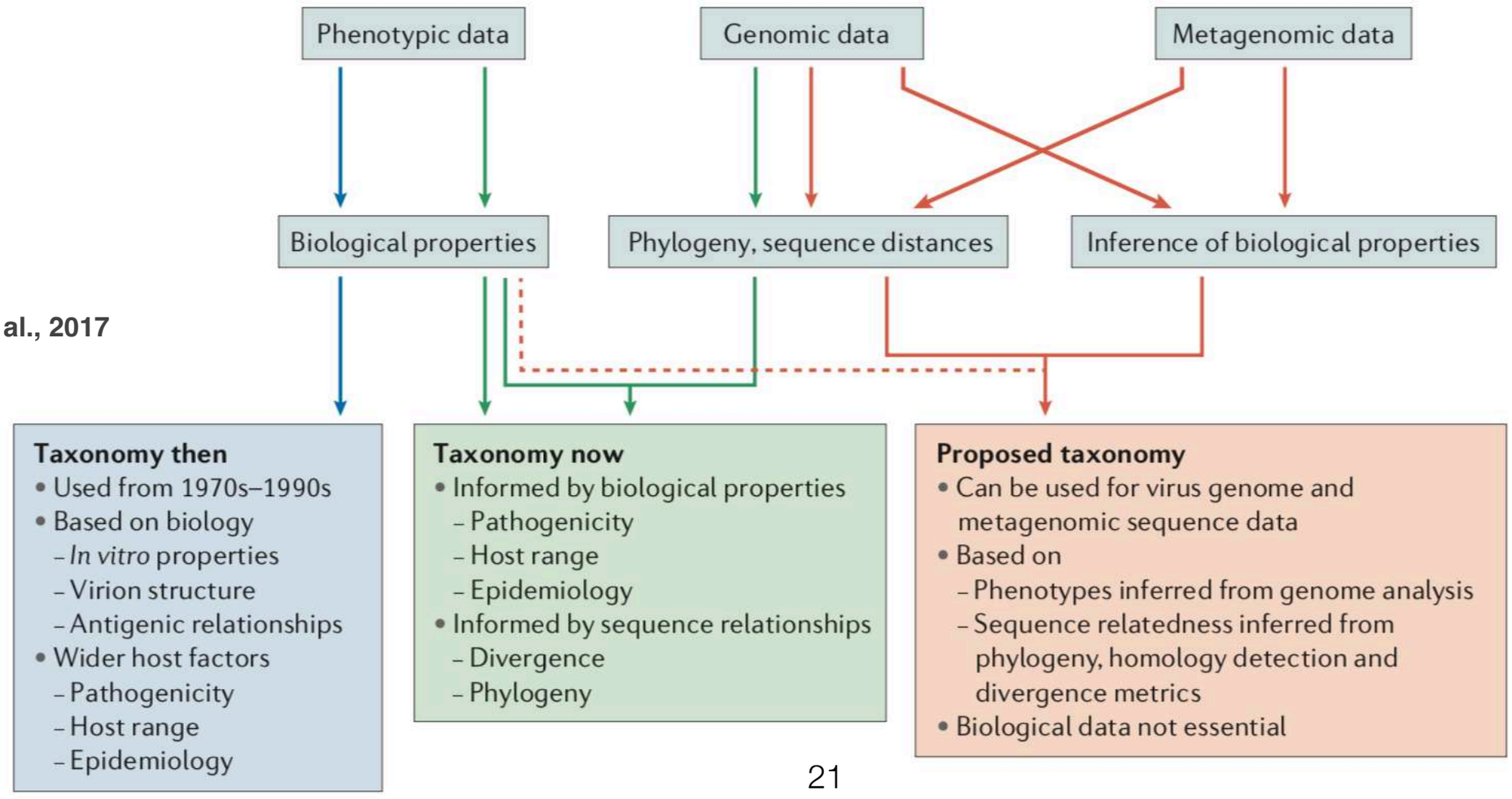
- Driver of viral evolution: mechanism for cells to quickly move genes about in nature
- **Viruses could have been selected as a means of enriching the genetic diversity and fitness of their hosts by facilitating gene transfers between them (HGT)**
- **Earliest viruses** were **primarily latent (lysogenic)** and **evolved lytic** capacities only **later** to more rapidly access new hosts

Taxonomy

Traditionally focused on viruses that cause disease in humans, domestic animals and crops, high-throughput sequencing of environmental samples —> large virome everywhere in the biosphere

At least 10^{31} virus particles exist globally at any given time in most environments (marine and freshwater habitats and metazoan gastrointestinal tracts) , in which the number of detectable virus exceeds the number of cells by 10–100-fold

To help conceptualize the sheer number of viruses in existence, their current biomass has been estimated to equal that of 75 million blue whales (approximately 200 million tonnes) and, if placed end to end, the collective length of their virions would span 65 galaxies



Simmons et al., 2017

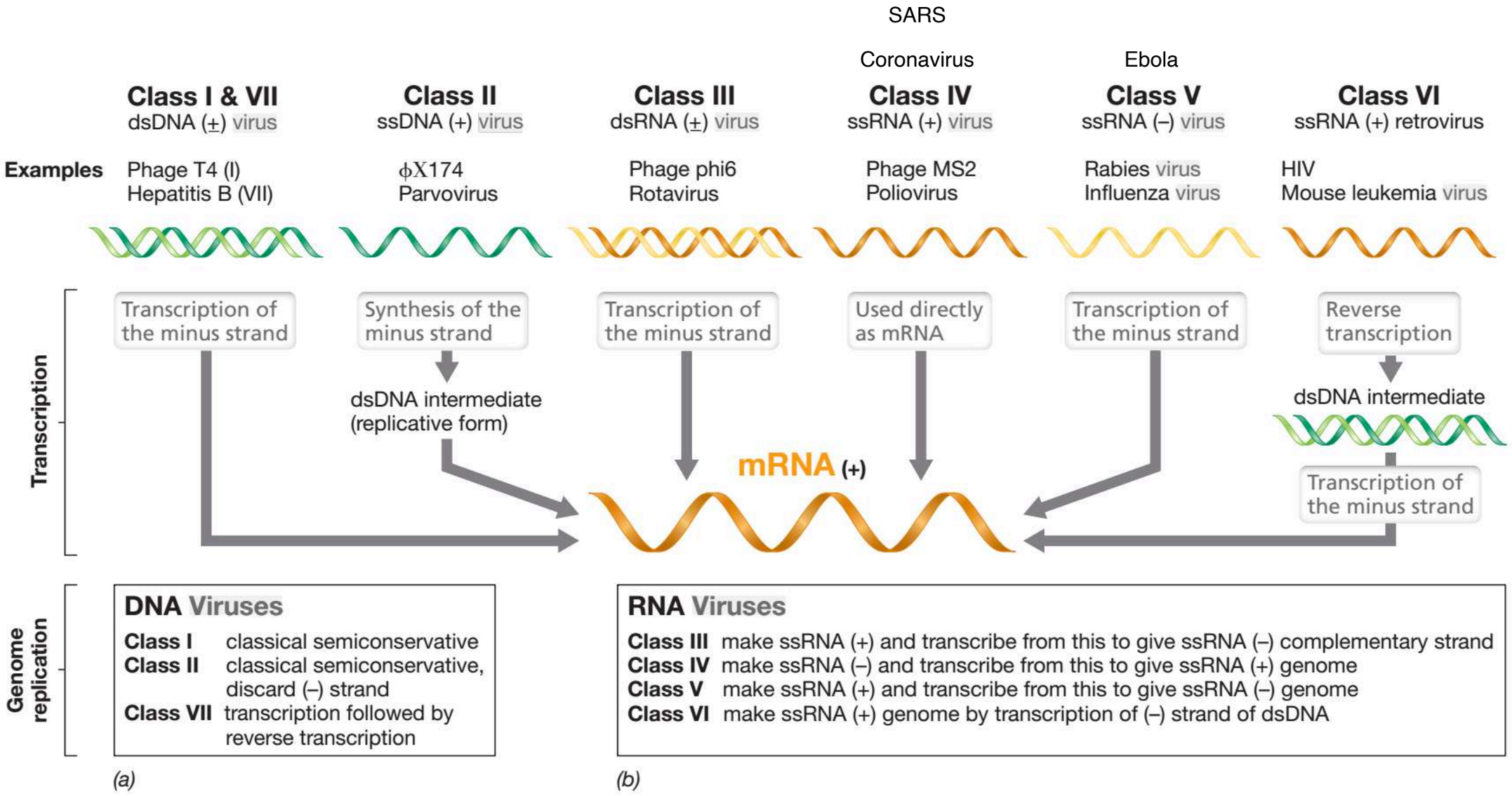
Nucleic acid content

"The Baltimore classification of viral genome"

SARS

Coronavirus

Ebola

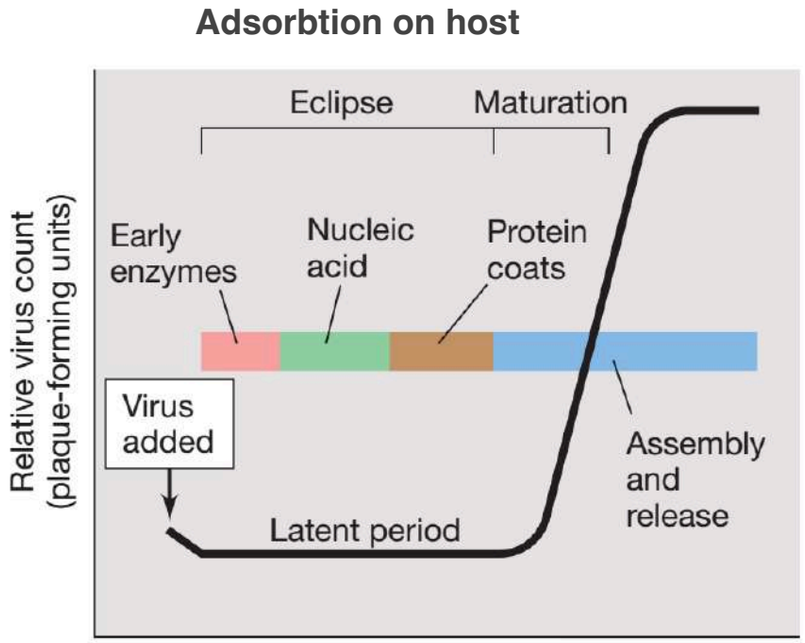


Madigan et al. 2020

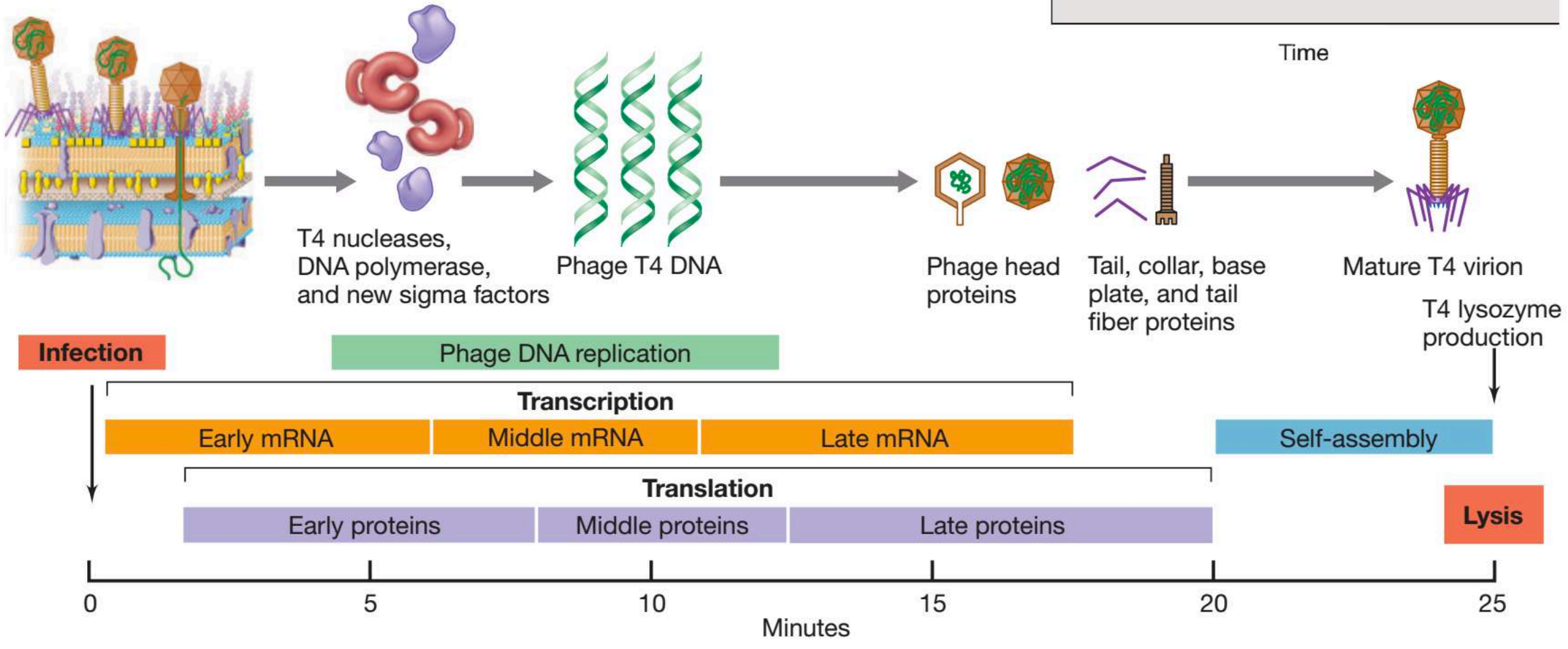
- Linear, circular & single stranded genomes
- Plus sense, minus sense in terms of their base sequence
- + configuration have = base sequence as host mRNA —> translation to form viral proteins
- - configuration are complementary in base sequence to viral mRNA

Replication cycle time course

- Nucleic acid content affects replication time
- Time is dictated by metabolic state of host cell and by what steps to produce plus complementarity mRNA from viral genome



Madigan et al. 2020



Madigan et al. 2020

Replication: attachment

- Viron itself has one or more proteins on its external surface interaction —> host cell receptors
- **Lack of specific receptor —> no infection**
- Receptors are functional machineries or part of structure:
  - Phage T1 —> **iron-uptake protein**
  - Bacteriophage lambda —> **maltose uptake system**
  - Bacteriophage T4 —> **carbohydrates in LPS**

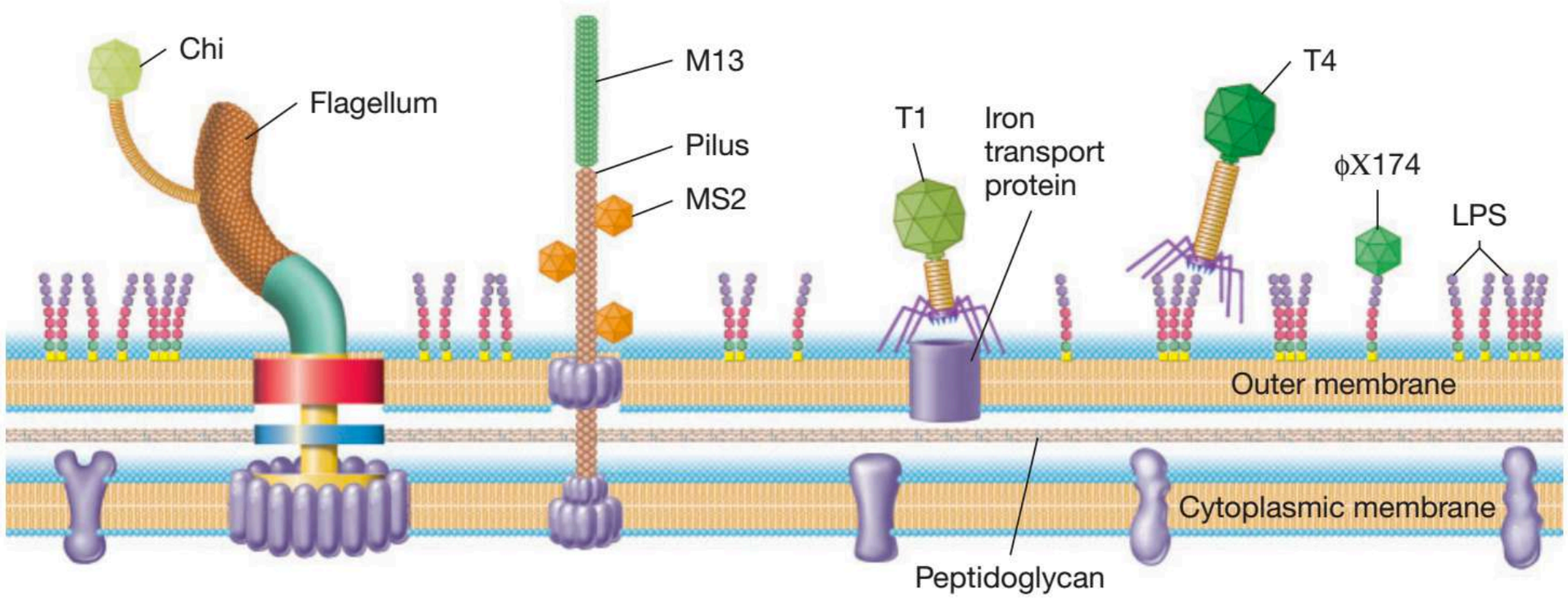
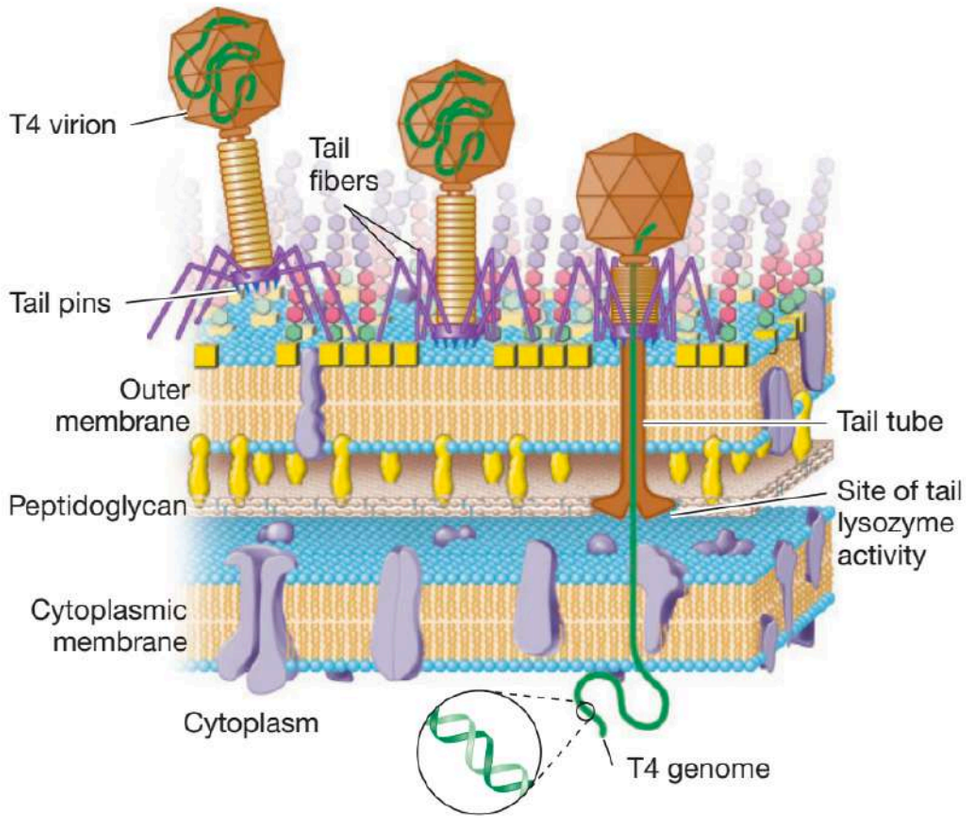


Figure 8.11 Bacteriophage receptors. Examples of the cell receptor sites used by different bacteriophages that infect *Escherichia coli*. All phages depicted except for MS2 are DNA phages.

Madigan et al. 2020

Replication: penetration, I

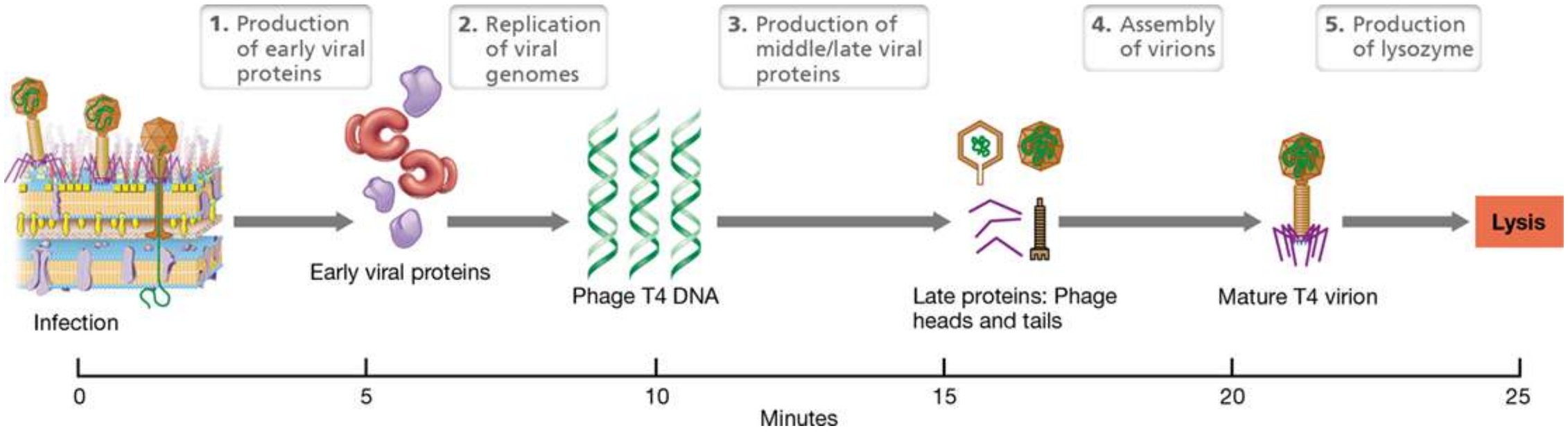
- Bacteriophage T4: lysosyme pore in peptidoglycan —> t**ail contracts like a syringe for injecting DNA (several minutes)**
- **Osmostic pressure** in host to be counterpoised ~50 atm (~ 500 m of water, picoNetwon forces) for injection (Evilevitch et al., 2003)



Madigan et al. 2020

Replication: penetration, II

- **Restriction endonucleases** can cleaved double strain **viral DNA if recognized**
- Viral DNA has **5-hydroxymethylcytosine in place of cytosine**



Madigan et al. 2020

Genome replication, transcription & translation, II

- Bacteriophage **T4 (double strain DNA) encodes its own DNA polymerase**

- **Transcription minus stand for mRNA**

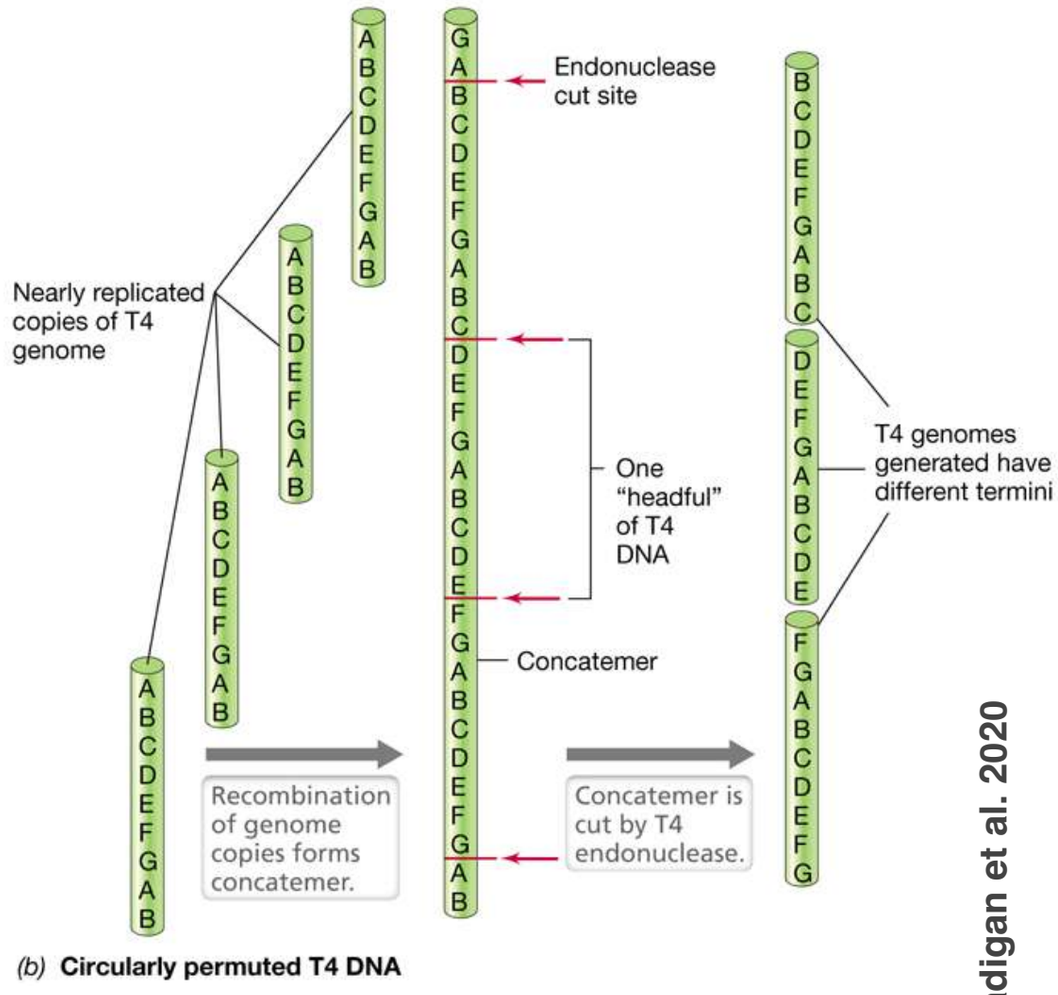
- In **T4 population** each copy of the genome contains the **same set of genes**, with **different arrangement** order —> circular permutation

- T4 population **genomes are terminally redundant**, meaning that some DNA sequences are duplicated on both ends of the DNA molecule

Genome replication, transcription & translation, II

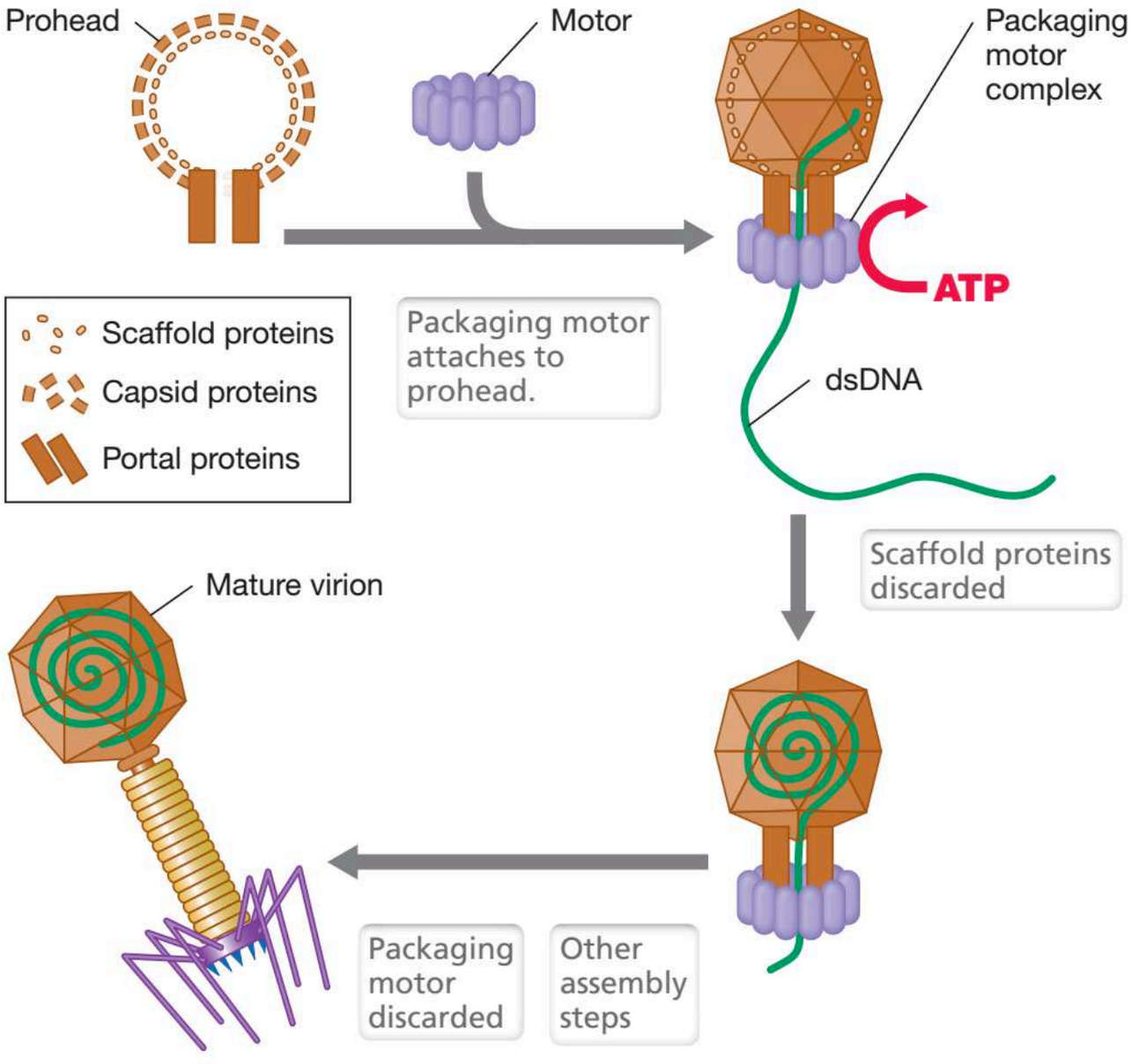
- T4 genome is first replicated as a unit and then **several genomic units are recombined end to end** to form a **long DNA** molecule called a **concatemer** ("two or more linear nucleic acid molecules joined covalently in tandem")

- T4 genome **does not encode its own RNA polymerase**; instead, **T4**-specific **proteins modify the specificity of the host RNA polymerase** so that it **recognizes only phage promoters**



Madigan et al. 2020

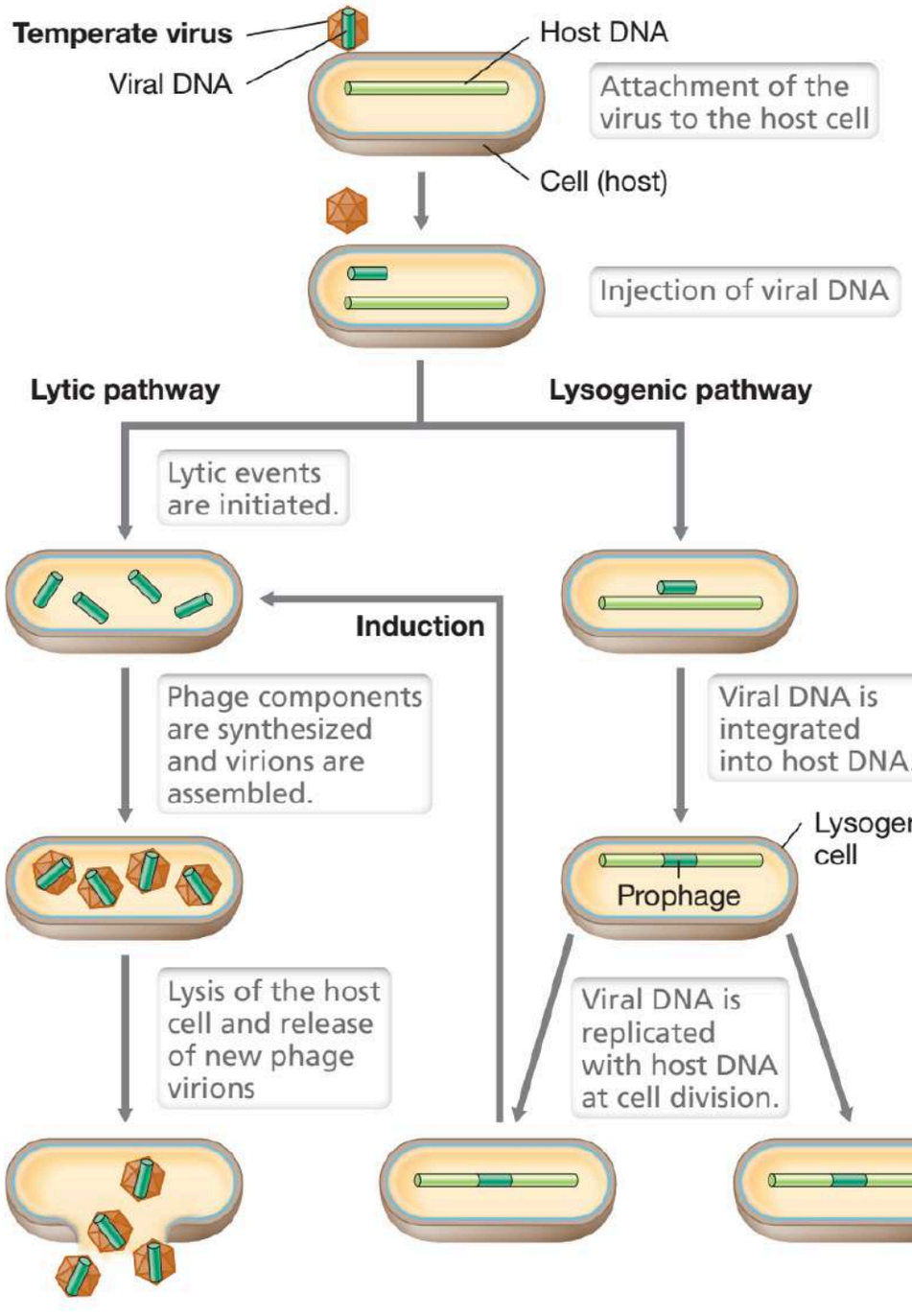
Replication: packaging, assembly & release



Madigan et al. 2020

- When the T4 **DNA is packaged** into capsids, the concatemer is not cut, is long enough to fill a phage head are generated (headful packaging)
- Generation **terminal repeats of about 3–6 kbp at each end of the DNA** molecule
- **ATP hydrolysis by a terminase** to push DNA into capsid, a packaging nanomotor
- Terminase can generate a force of up to **100 pN**
- ~ 50 atm to counterpoise
- **Tight packaging, ~500 mg per ml**

Temperate bacteriophage and lysogeny

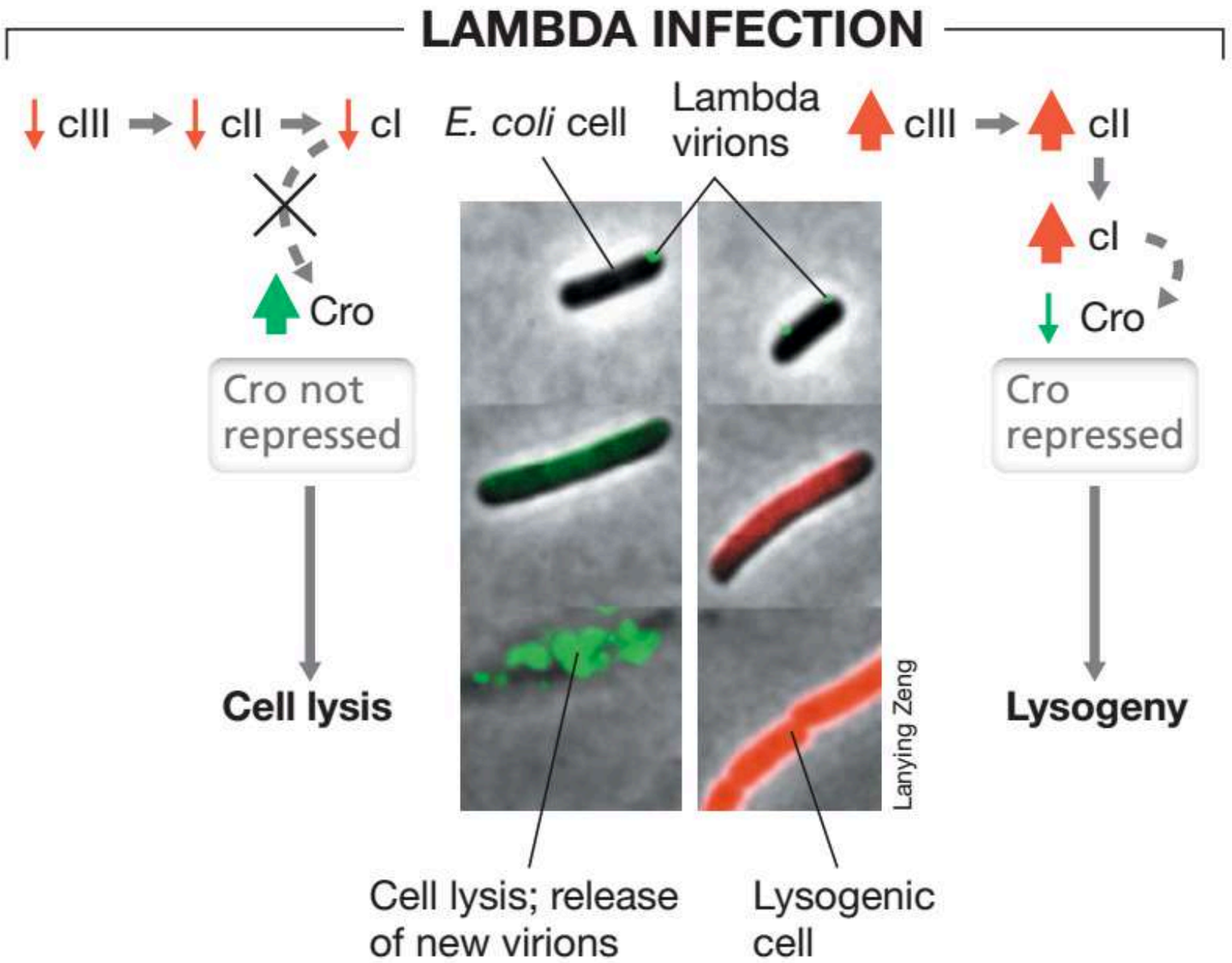


Madigan et al. 2020

- During lysogeny, the temperate virus genome is either integrated into the bacterial chromosome (lambda) or as a plasmid (P1)
- Viral DNA, now called a prophage, replicates along with the host cell as long as the genes that activate the phage virulent pathway are repressed
- Maintenance of the lysogenic state is due to a phage-encoded repressor protein
- Host under nutrient stress induce the switch

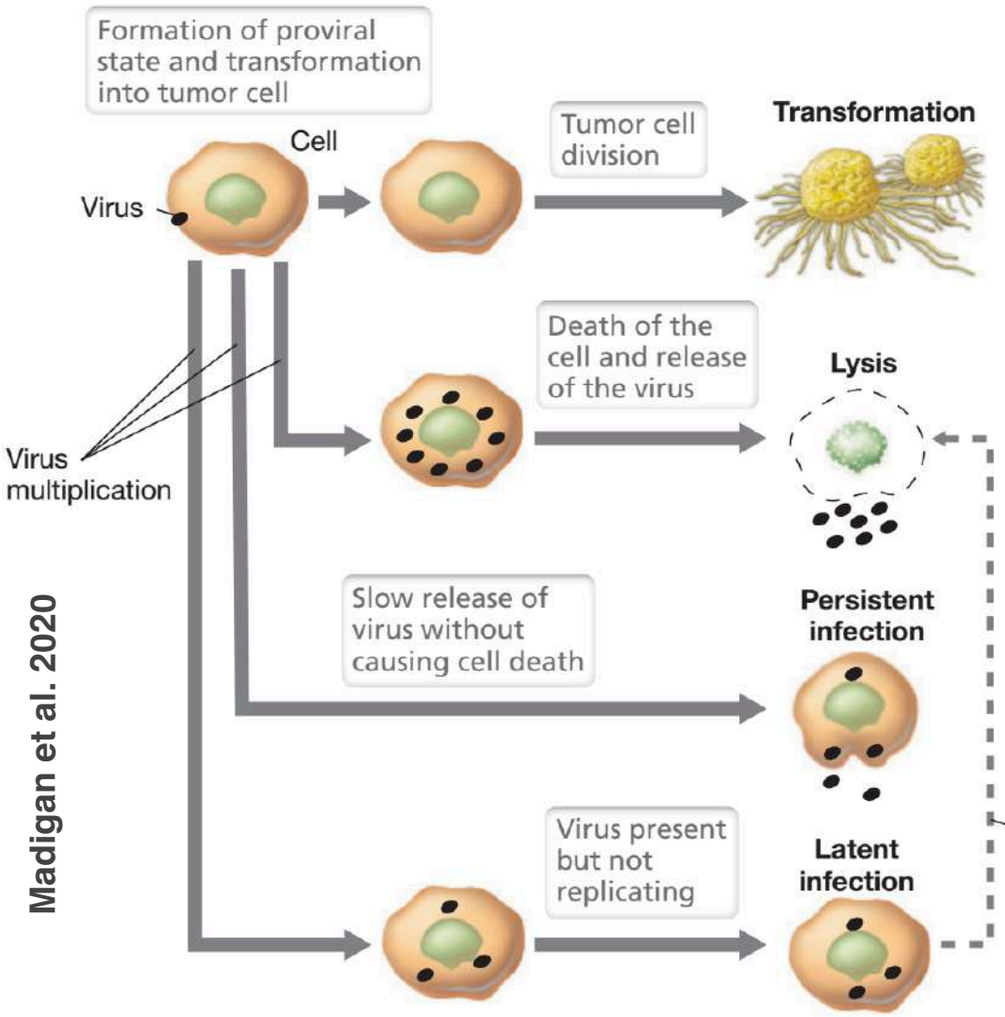
Genetic switch for decision making

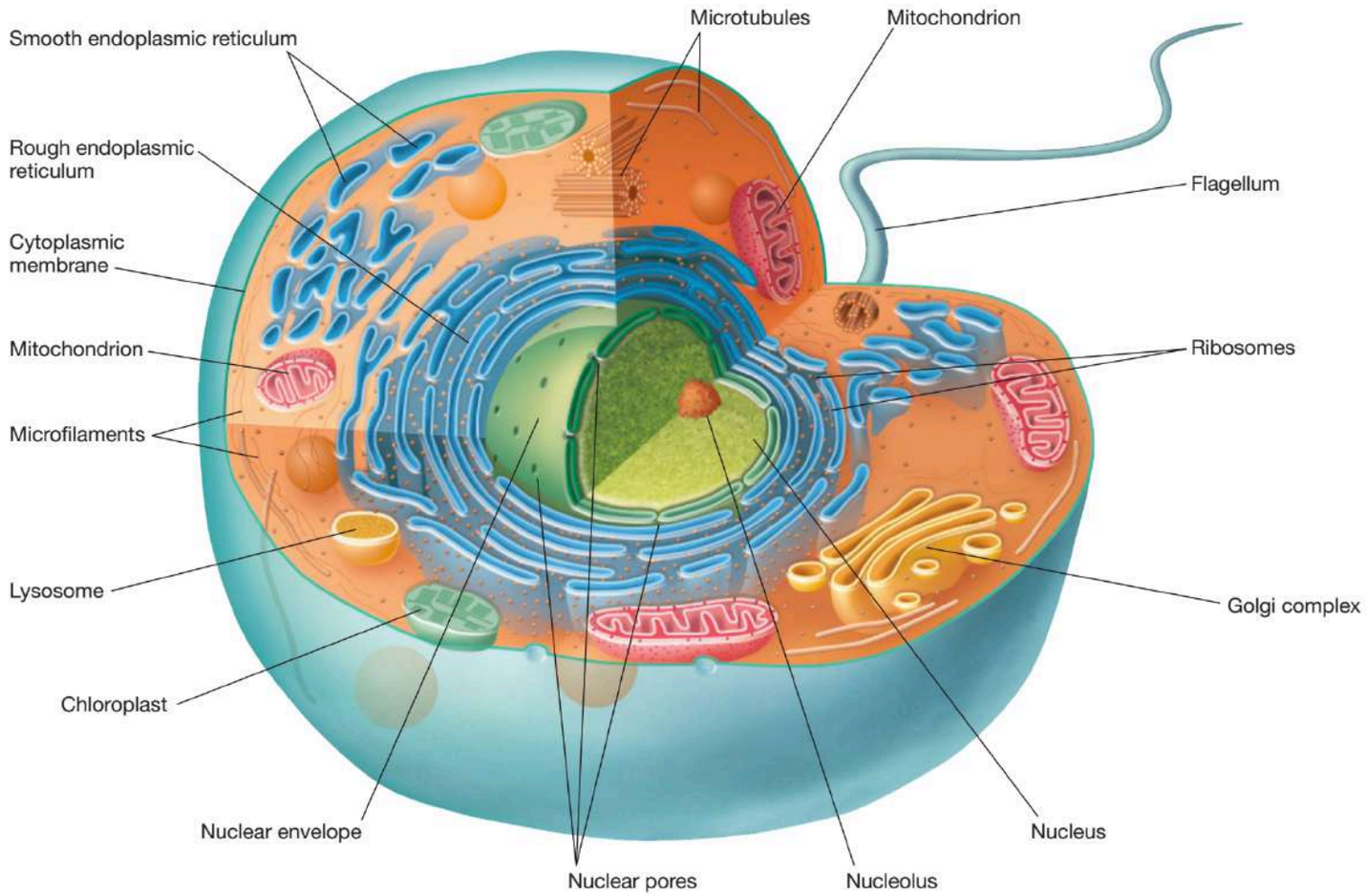
Double repressor interplay dictate the fate of the Lambda phage infection



Madigan et al. 2020

Viruses infecting Eukaryotes (animal cells)



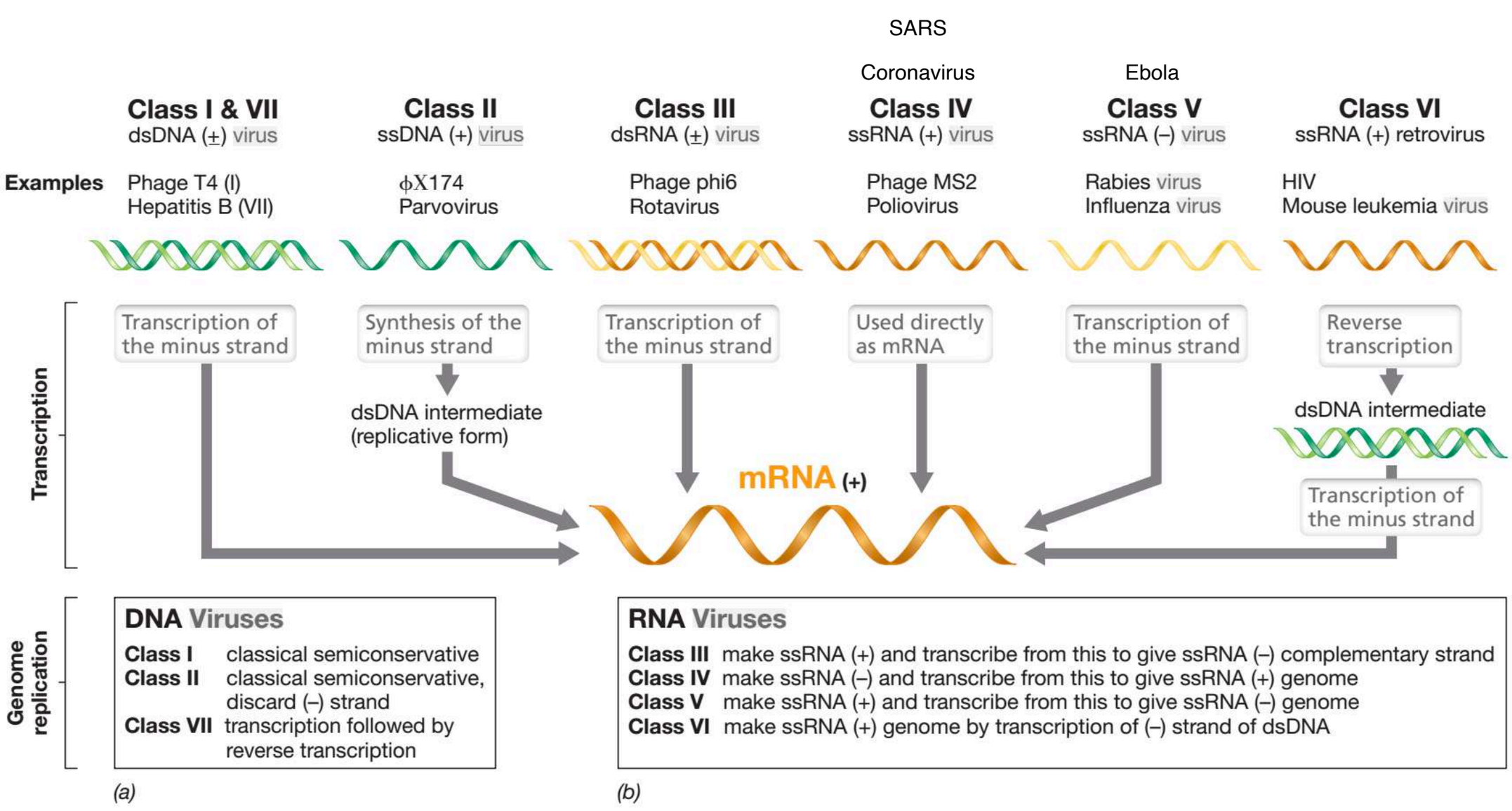


Eukaryote:

Defined **nucleus** with nuclear membrane genomes structured in chromosomes-bodies containing the hereditary material)

Organelles: **mitochondria** (cellular energy, oxidation); **hydrogenosomes** (fermenative metabolism); **Golgi apparatus** (secretory device); an **endoplasmic reticulum** (a canal-like system of membranes within the cell for protein, lipid synthesis); **lysosomes** (digestive apparatus within many cell types); **chloroplast** (glucose and ATP production and O_2 in plants); **cytoskeleton** (3D structural architecture); **flagella** and **cilia**

Viruses infecting Eukaryotes exploit diverse genome architecture



Madigan et al. 2018

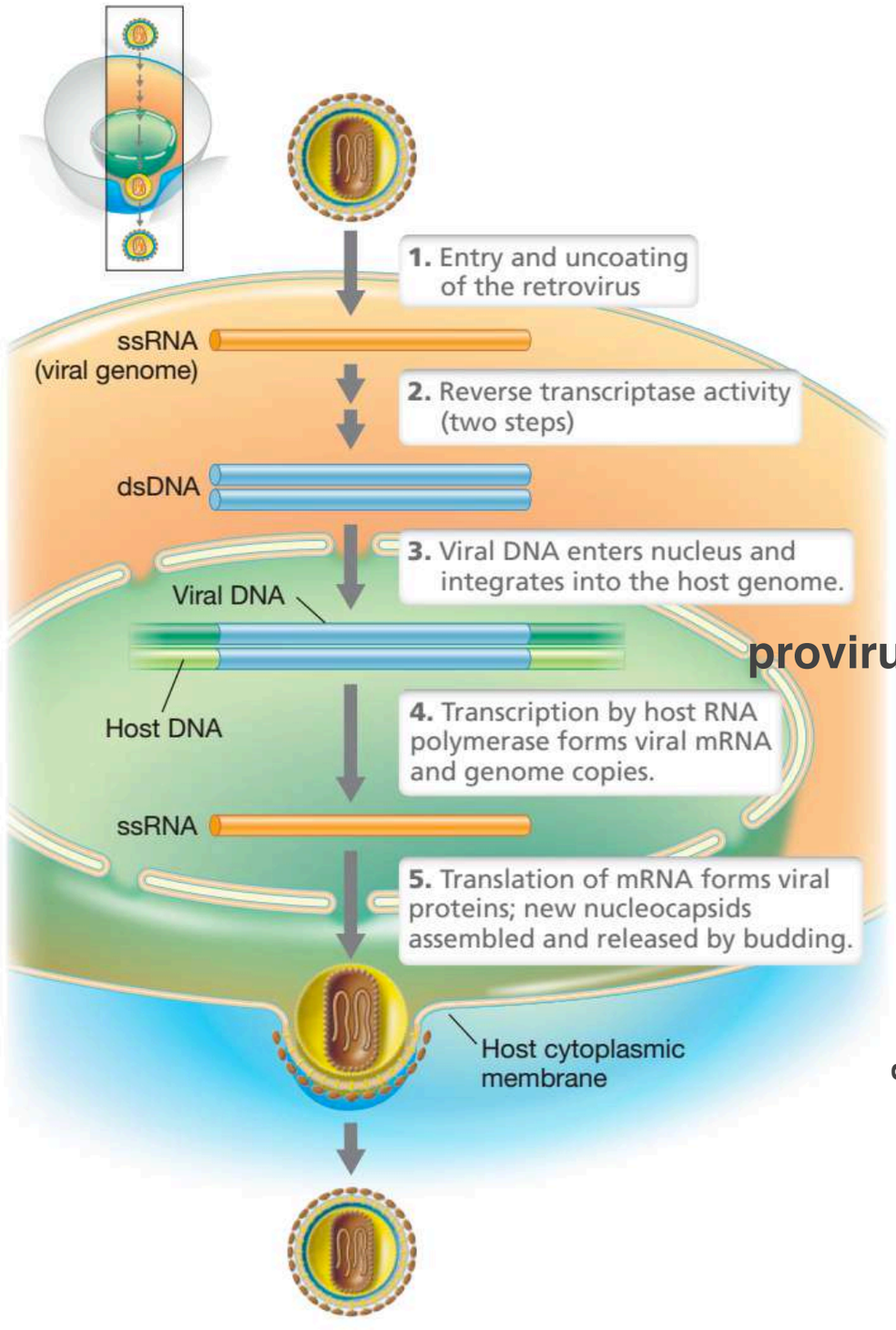
Breaking the dogmas:

1. DNA —> RNA flow of information

2. DNA —> RNA —> Proteins

A new dogma in the flow of information: Retrovirus

- Retroviruses contain an RNA genome, SS RNA (+)
- **RNA genome is replicated inside the host cell by way of a DNA intermediate**
- Retroviruses **transfer** information **from RNA —> DNA** (in contrast to genetic information flow in cells, which occurs from DNA —> RNA)
- **Reverse transcriptase** enzyme present in virus and start transcribing once in cytoplasm
- Retroviruses cause cancer and acquired immunodeficiency syndrome (AIDS)



Madigan et al. 2018

Subviral agents: high diversity in the viriosphere

- Virus: the word is from the Latin **virus referring to poison** (Beijerinck)
- Virus-like transmissible agents
- Transmissible, pathogenic to their host, and filterable
- Three kinds:
- **Satellite viruses - replication depends on another virus** (i.e. a host virus) —> “a parasite of parasite”
- **Viroids** - small RNA molecule (~0.3 kb circular RNA) only, but is devoid of proteins
- **Prions** - that are associated with *TSE* (transmissible spongiform encephalopathy) or scrapie composed of **proteins only**, but devoid of nucleic acids **breaking another dogma Protein —> DNA**

Wang-Shick, 2017

Features	Satellite Virus	Viroid	Prion
Genome (Nucleic acid)	○	○	X
Protein coding	○	X	X
Particle protein	○ (Capsid)	X	○ (PrP)

Viroids and Prions

Viroids

Madigan et al. 2020

Diener et al., 1982

Viroid is **inactivated by ribonuclease** digestion, Zn^{2+}-catalyzed hydrolysis, and chemical modification with NH_2OH

Viroid **RNA** is a **single-stranded, covalently closed circle**, its extensive secondary structure forms a hairpinshaped double-stranded molecule with closed ends

Viroids are non-coding circular RNA molecules with rod-like or branched structures

They are often ribozymes, characterized by catalytic RNA

They can perform many basic functions of life and may have played a role in evolution since the beginning of life on Earth

They can cleave, join, replicate, and undergo Darwinian evolution

Prions

Prion agent was **inactivated by proteinase** K and trypsin digestion, chemical modification with diethylpyrocarbonate, and by exposure to phenol, NaDodSO4, KSCN, or urea

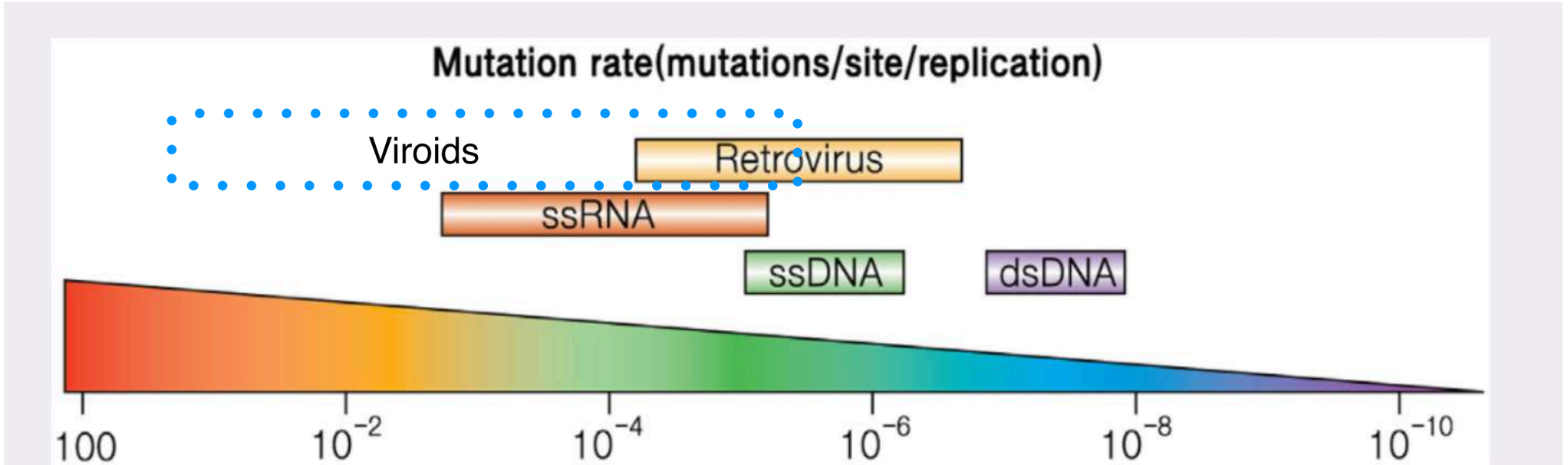
The host contains a **gene**, Prnp (Prion protein), which **encodes the native form of the prion**, known as PrPC (Prion Protein Cellular)

The **pathogenic prion** protein is designated PrPSc (prion protein **Scrapie**)

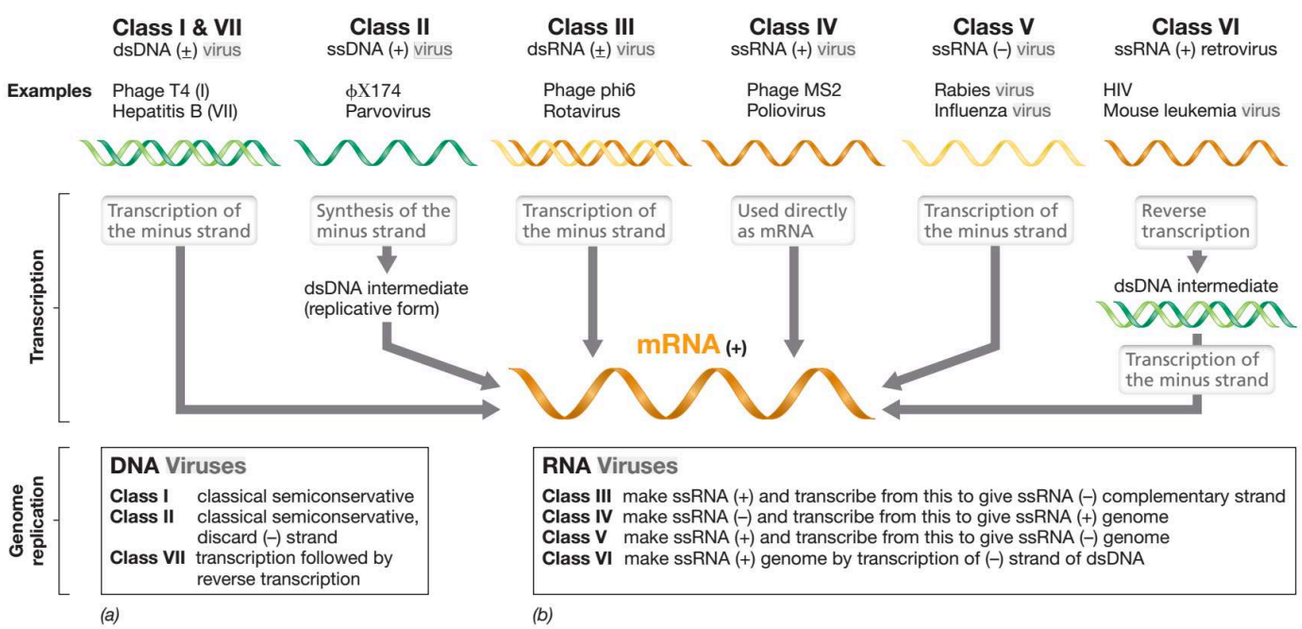
When the PrPSc form **enters** a host cell that is expressing PrPC, it **promotes the conversion of PrPC into the pathogenic form —> misfolding**

As the pathogenic prions **accumulate and aggregate** —> form **insoluble crystalline fibers** referred to as **amyloids** in neural cells

High mutation rate in the viriosphere



Wang-Shick, 2017



- **Mutation Eukaryotic rates are lower (10^{-8} - 10^{-10}) < virus**
- **Proofreading capability (10^{-3}) takes largely care of most of the errors**
- **RNA more flexible than DNA**
- **In accordance with evolution theories**
- **If viruses integrate their mutation rate depends on host mutation rates**