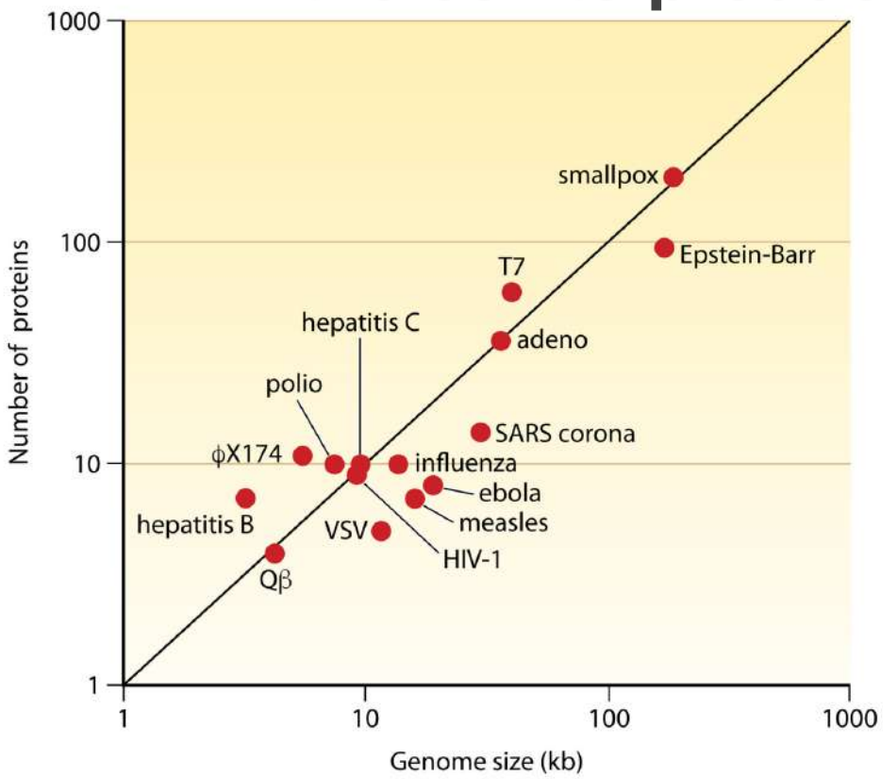
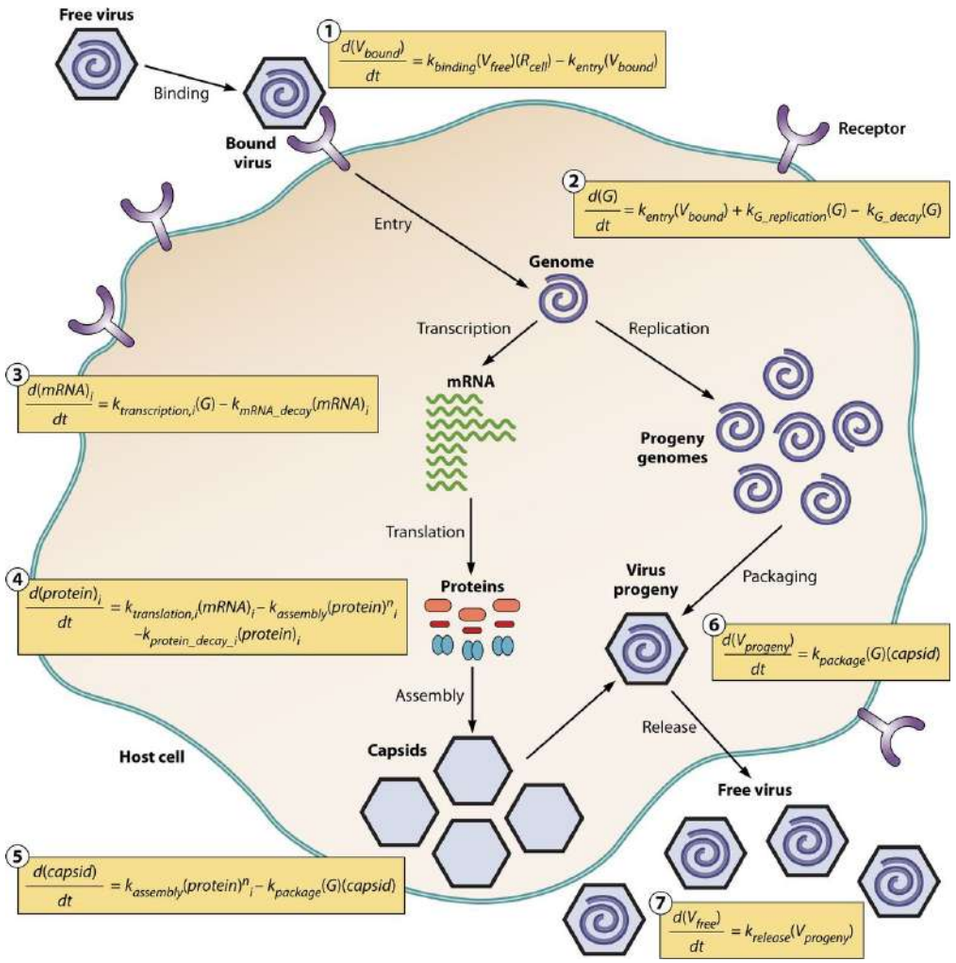
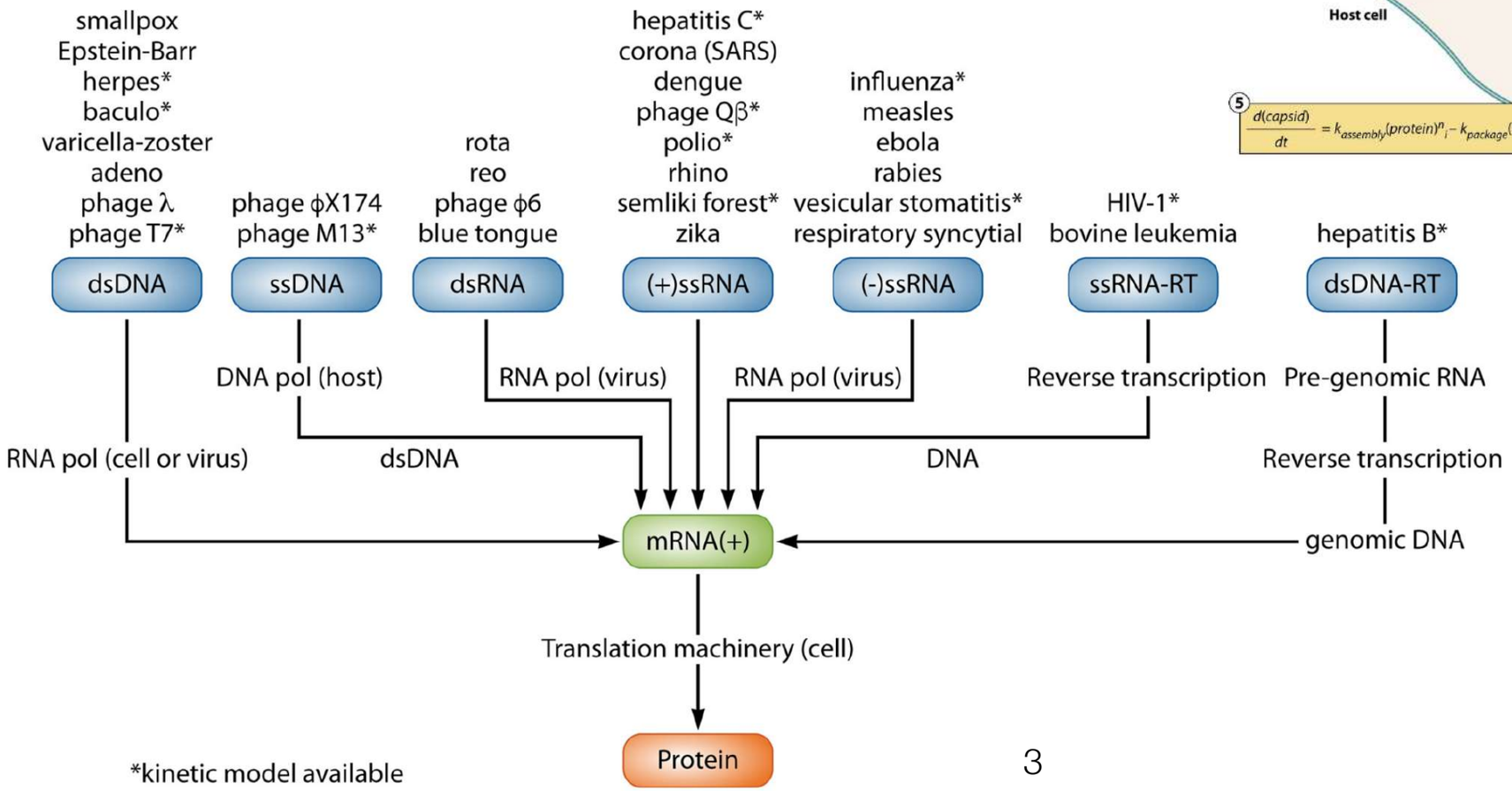
LO4b

Recap

When a virus infects a host cell, it hijacks the biosynthetic capacity of the cell to produce virus progeny (< 1 hr and > wks)

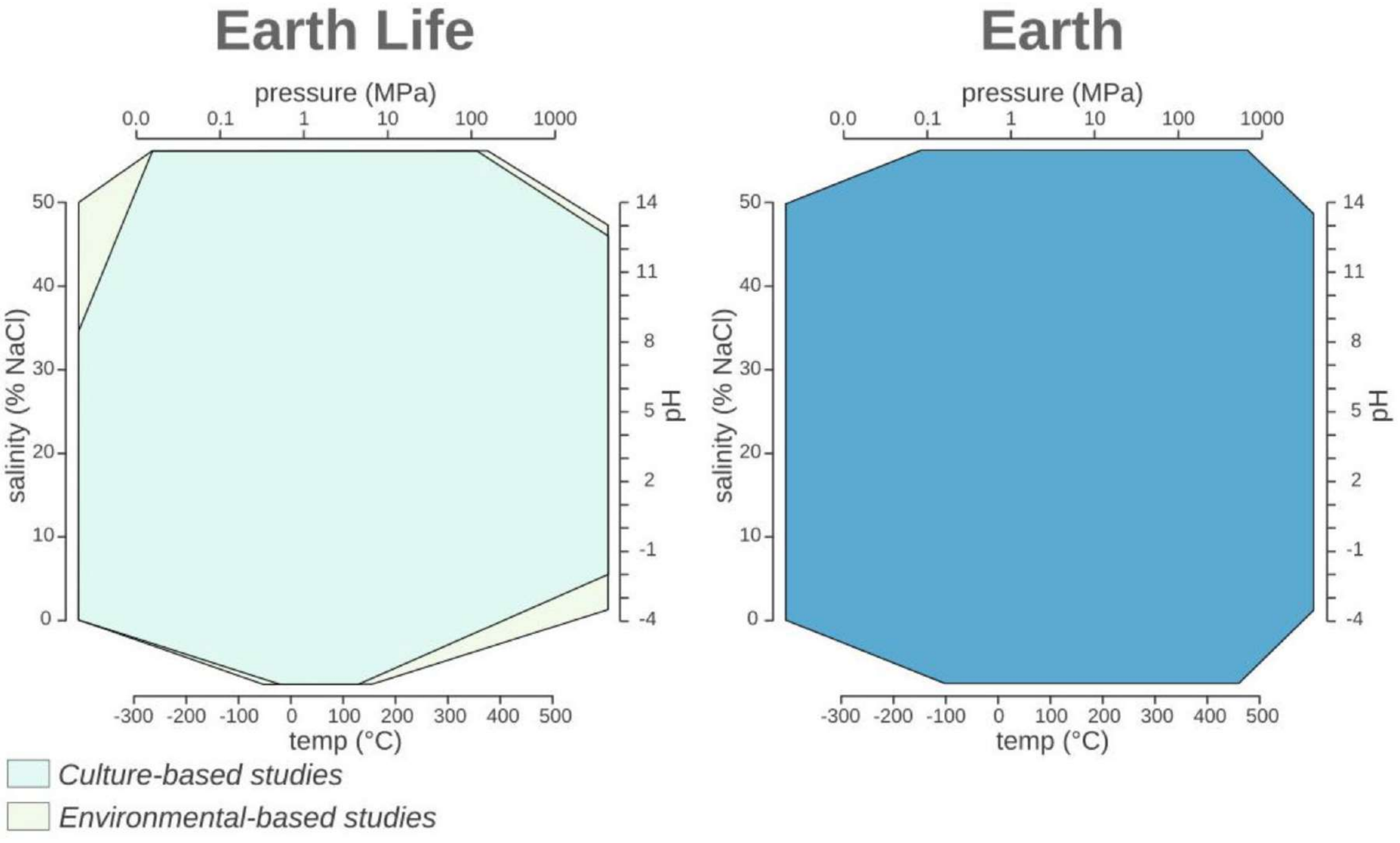






Yin & Redovich, 2018

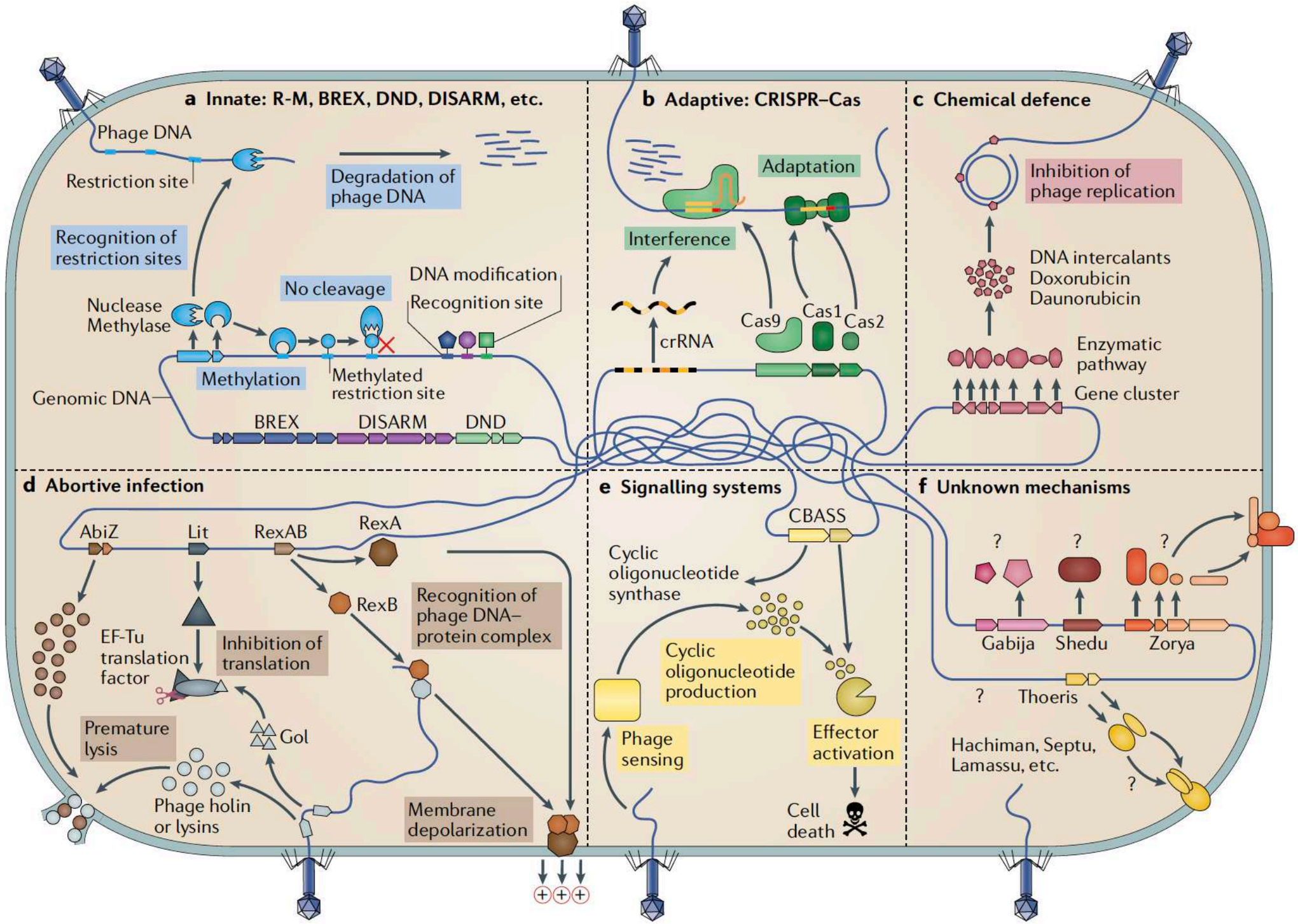
Archaeal viruses



Merino et al. 2019

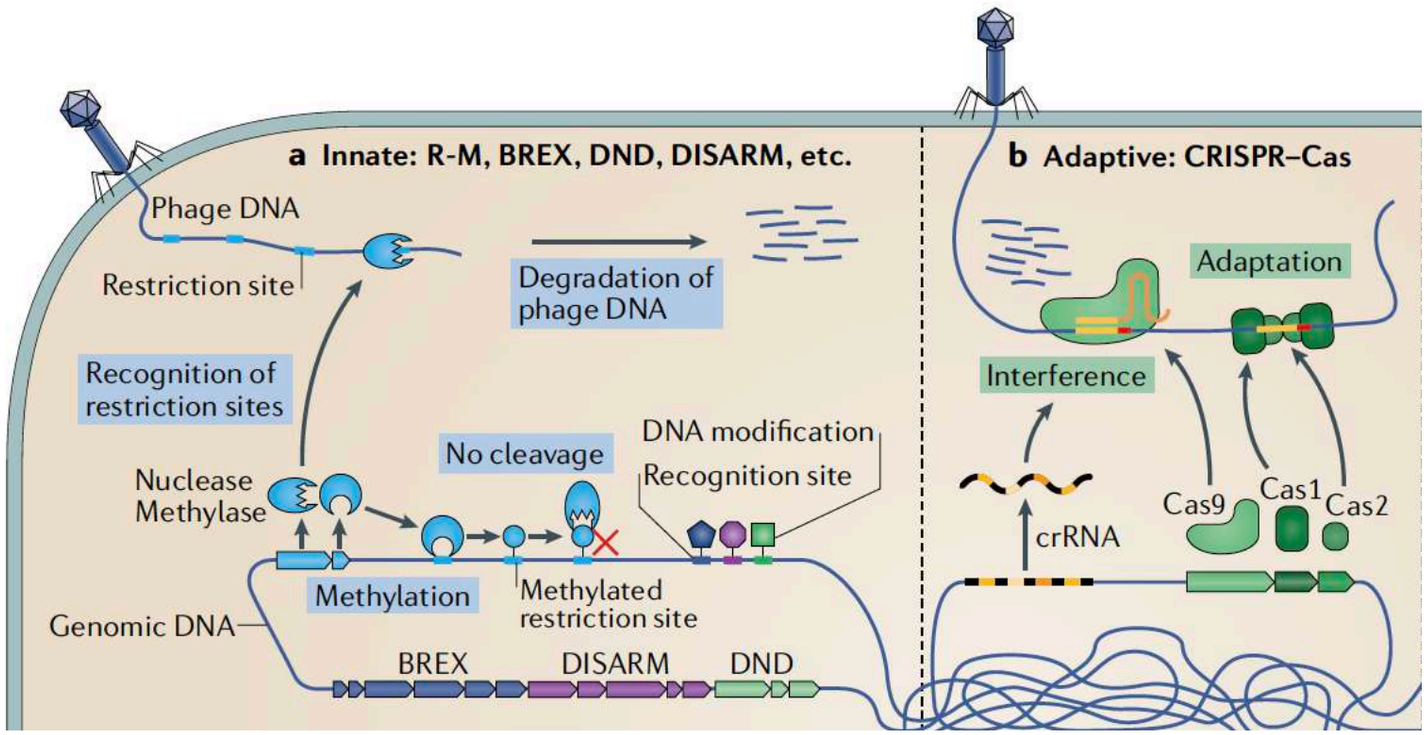
Common, not much known, in extreme environment

Antiviral defence systems in bacteria



Bernheim & Sorek, 2020

Antiviral defence systems in bacteria



Bernheim & Sorek, 2020

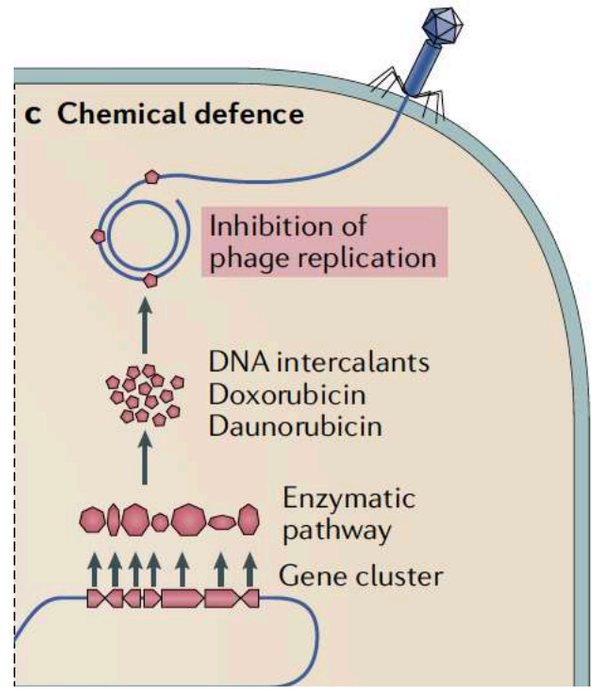
Defence systems that target nucleic acids encompass both innate and adaptive immunity

a | Restriction-modification (R-M) and other related systems modify specific sequence motifs in the host genome and cleave or degrade unmodified foreign DNA

b | CRISPR–Cas systems work in two main phases: adaptation, where a complex of Cas proteins guides the acquisition of new bacteriophage (phage)-derived spacers; and interference, where Cas proteins in a complex with a spacer-derived CRISPR RNA (crRNA) target and degrade phage nucleic acids

Antiviral defence systems in bacteria

C, Chemical defence has been described in *Streptomyces* app. In which bacteria produce a small anti-phage molecule that intercalates into phage DNA and inhibits its replication



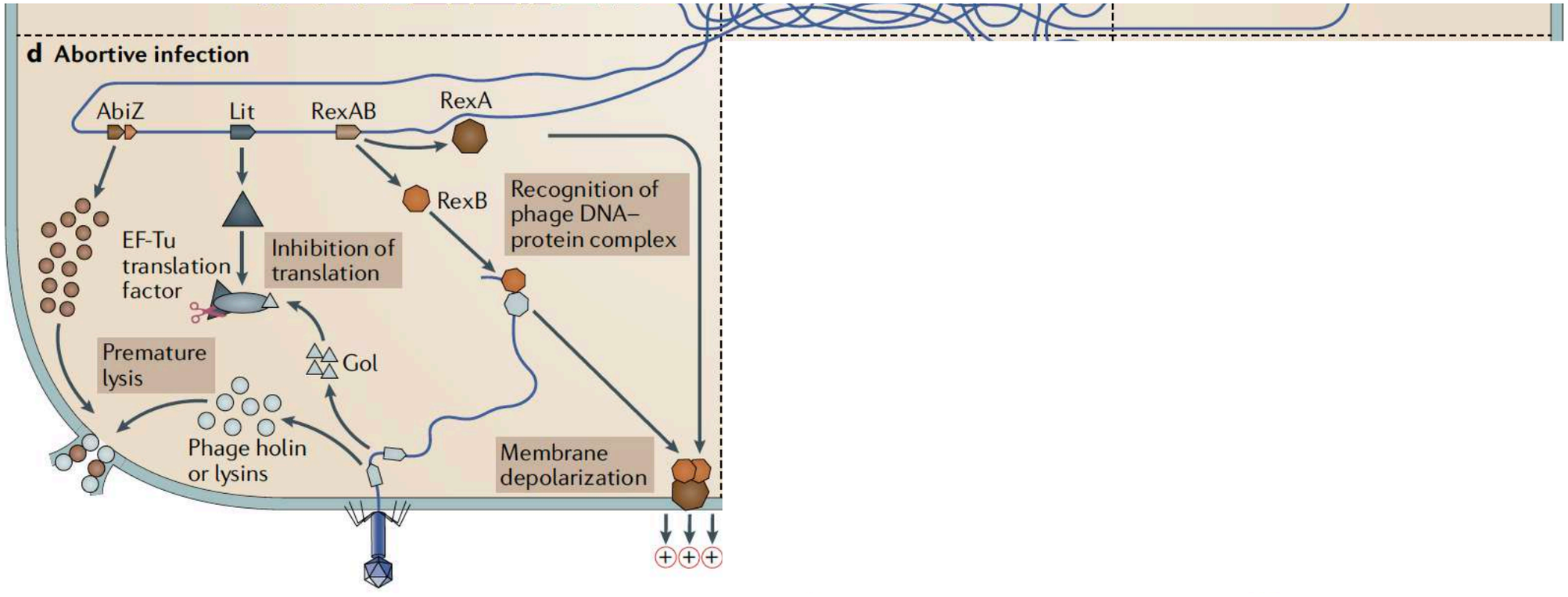
Bernheim & Sorek, 2020

Antiviral defence systems in bacteria

Abortive infection mechanisms are diverse. In concert with phage-encoded holins and lysins of phage Phi31, AbiZ from *Lactococcus lactis* accelerates lysis before phage assembly is completed.

Upon expression of the T4 phage protein Gol, the *Escherichia coli* Lit protein inhibits translation through cleavage of the EF-Tu elongation factor

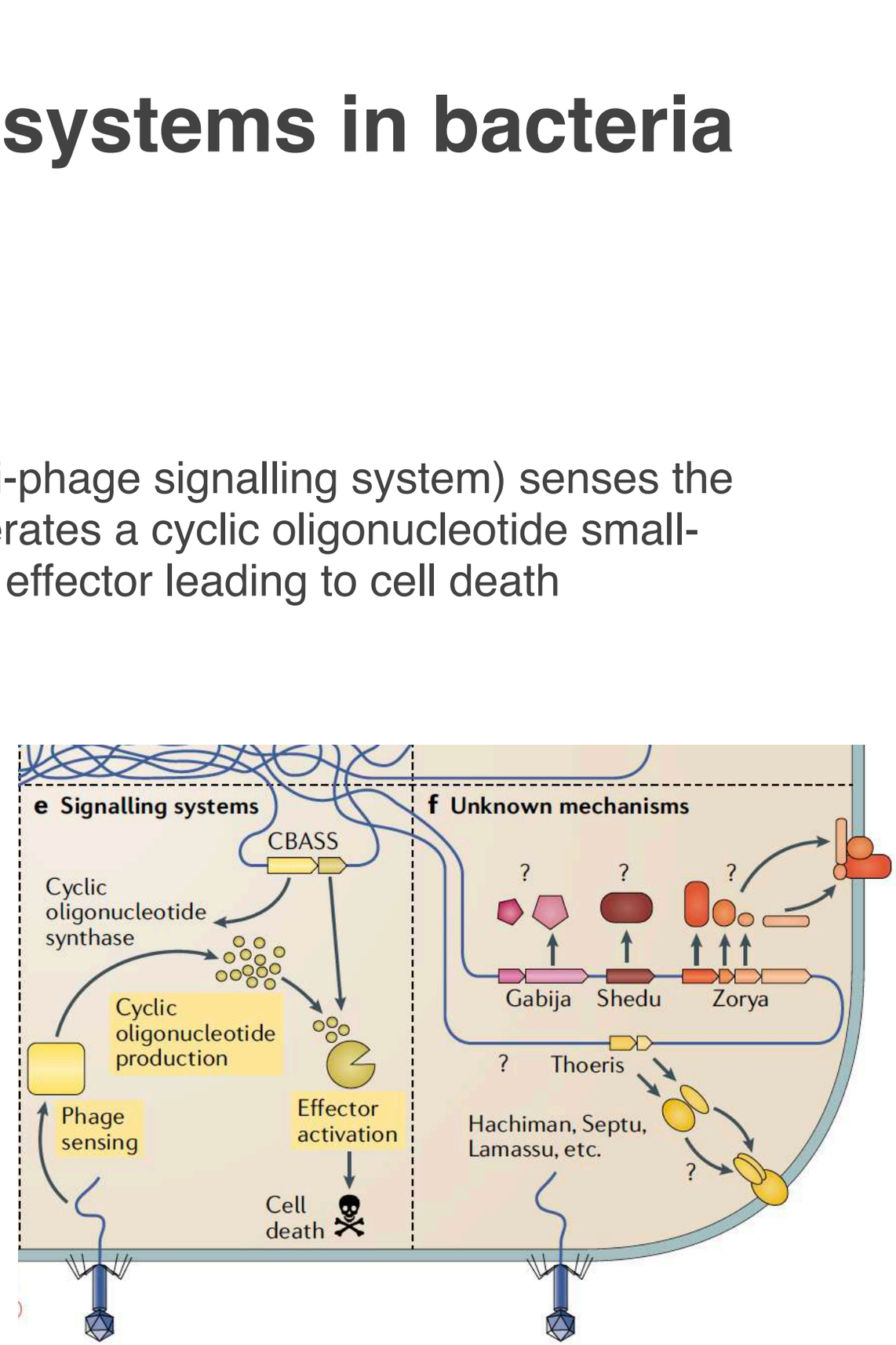
The *E. coli* protein RexA recognizes a specific DNA–protein complex formed by the λ phage, and activates RexB, an ion channel that depolarizes the membrane, leading to cell death.



Bernheim & Sorek, 2020

Antiviral defence systems in bacteria

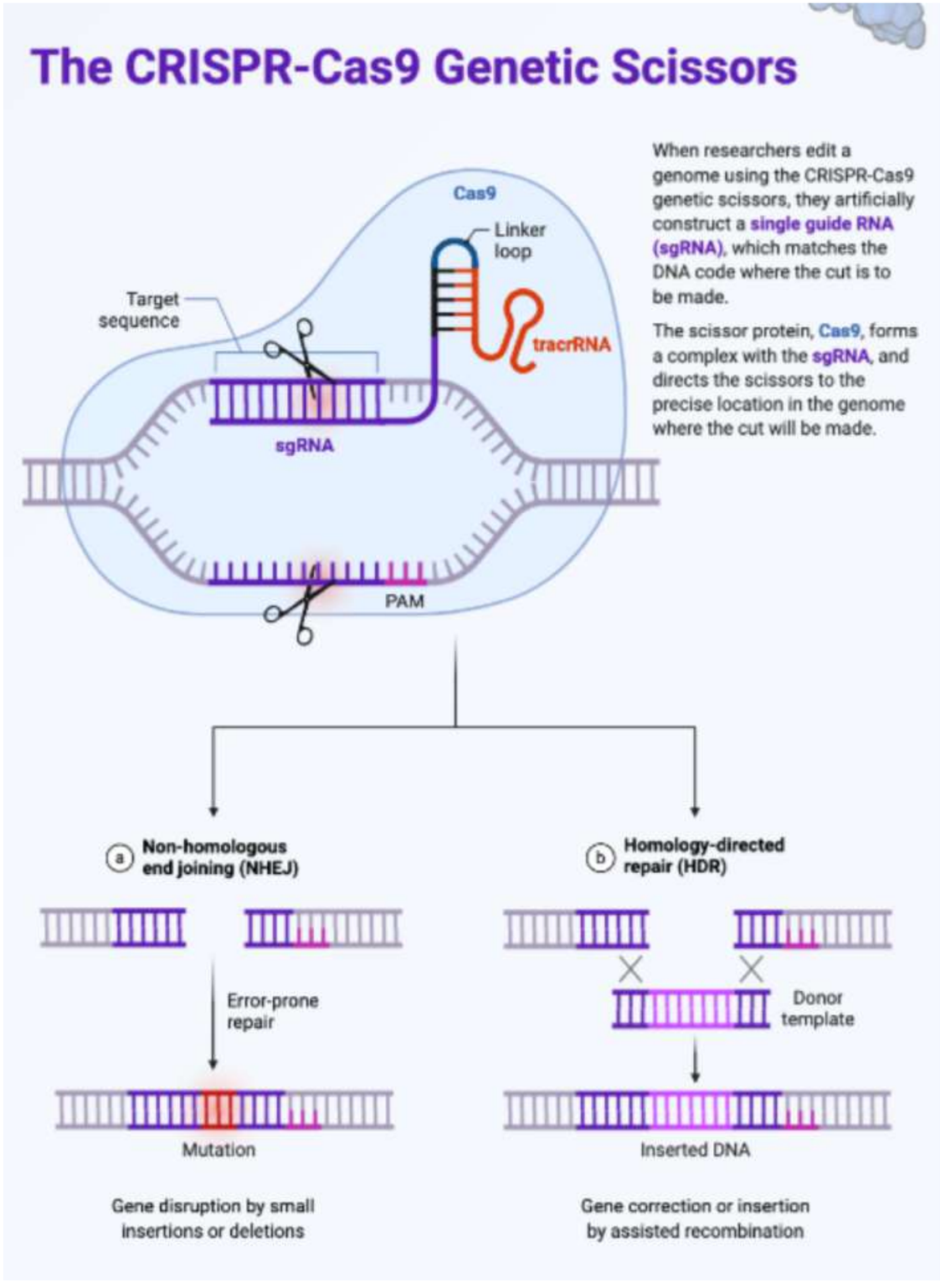
CBASS
(cyclic oligonucleotide-based anti-phage signalling system) senses the prespresence of phage and generates a cyclic oligonucleotide small-molecule signal that activates an effector leading to cell death



Bernheim & Sorek, 2020

CRISPR, the clustered regularly interspaced short palindromic repeats

CRISPR is the simplest and most versatile method for editing the genome sequence in living organisms to date

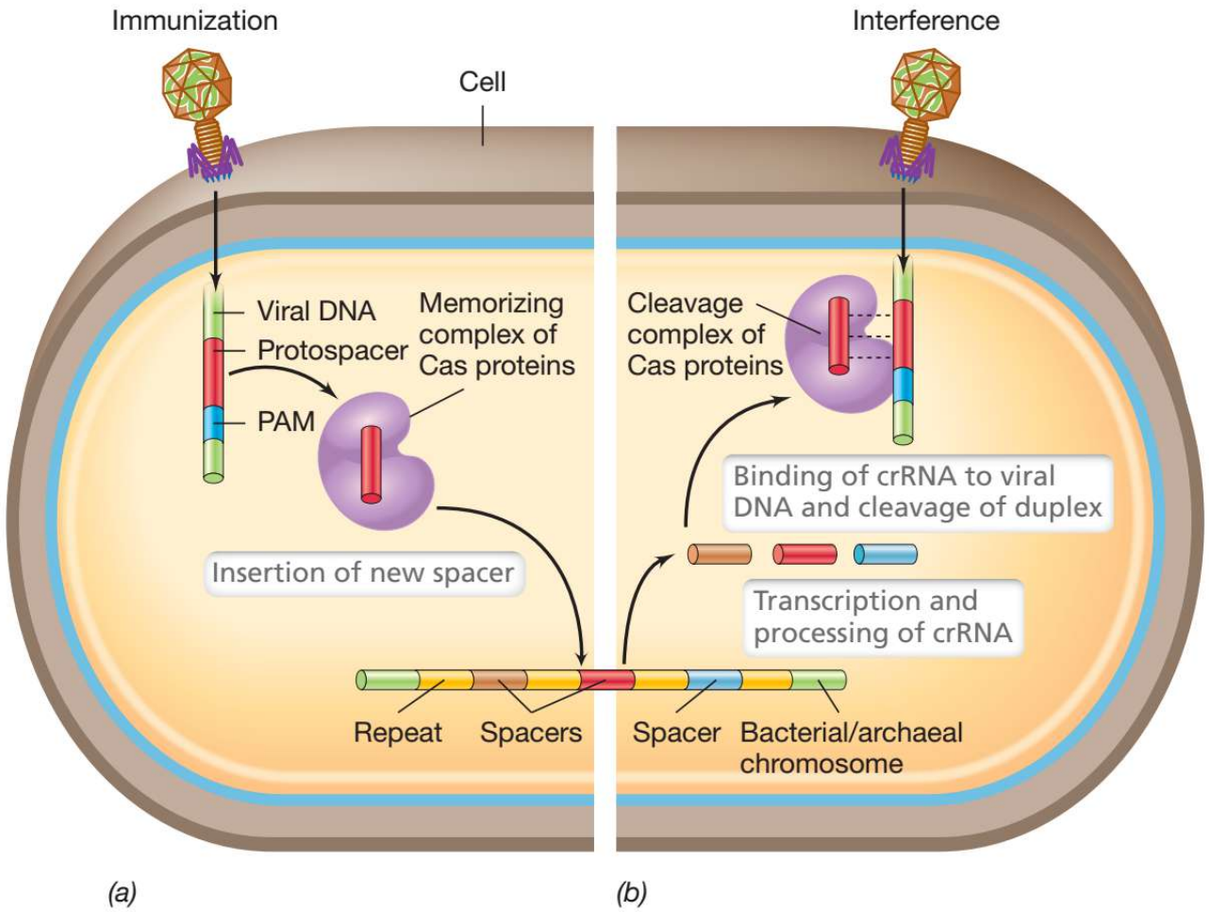


A Nobel Prize for genetic scissors. Nat. Mater. 20, 1 (2021). https://doi.org/10.1038/s41563-020-00895-z

https://app.biorender.com/biorender-templates/figures/5f8f6662269fc400282cbda4

CRISPR, the clustered regularly interspaced short palindromic repeats, I

Immunization



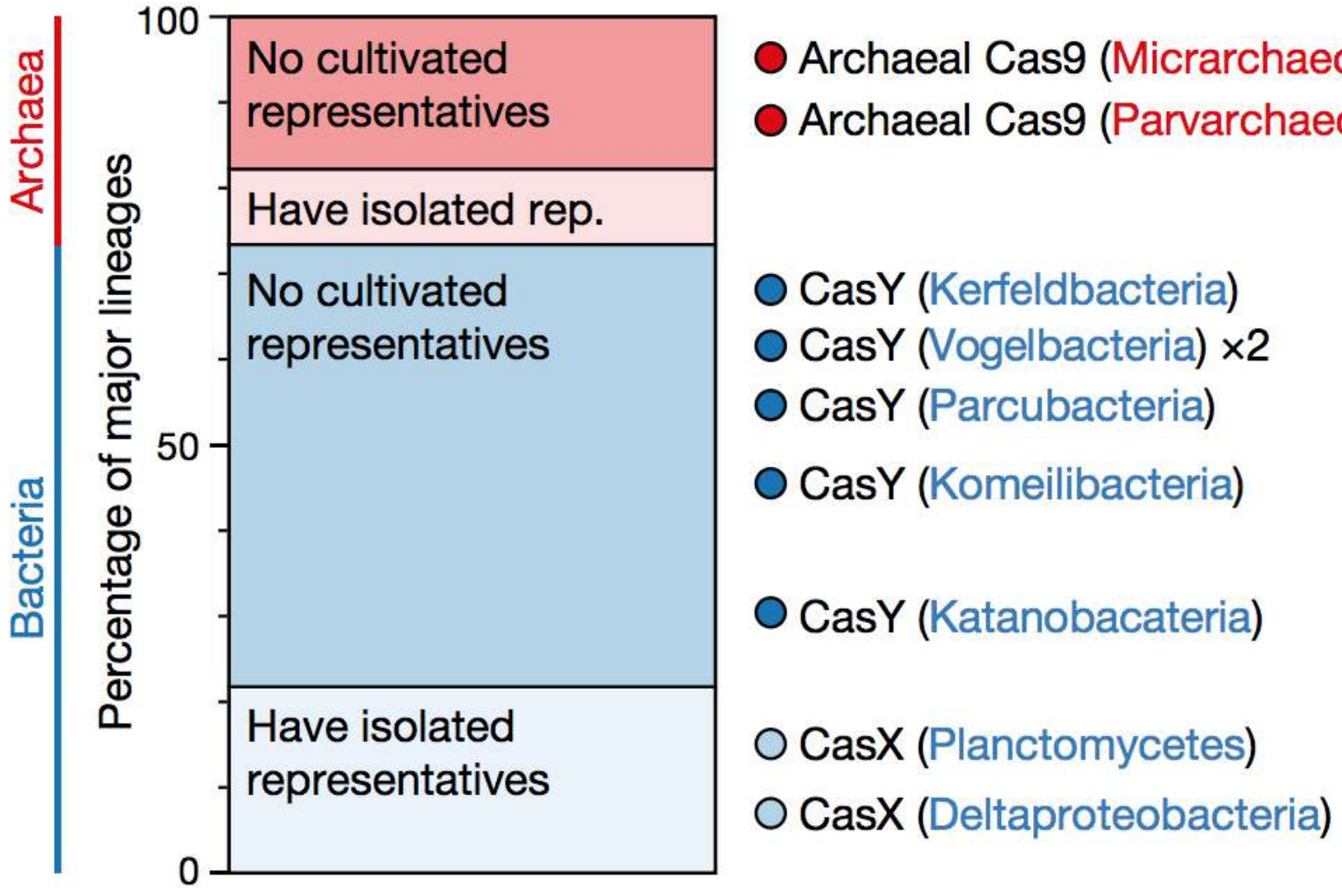
Interference

Madigan et al. 2020

- Antiviral system in Bacteria & Archaea
- CRISPR contains **short repeats of constant DNA** sequence **alternating** with short **variable DNA sequences, spacers**
- **Spacers** are pieces of **viral or other foreign DNA** and function as "**memory bank**" of past viral encounters
- **Cas** (CRISPR-associated) **proteins** have **endonuclease** activity for the **defense against foreign DNA** and **incorporate new spacer regions** into CRISPR region

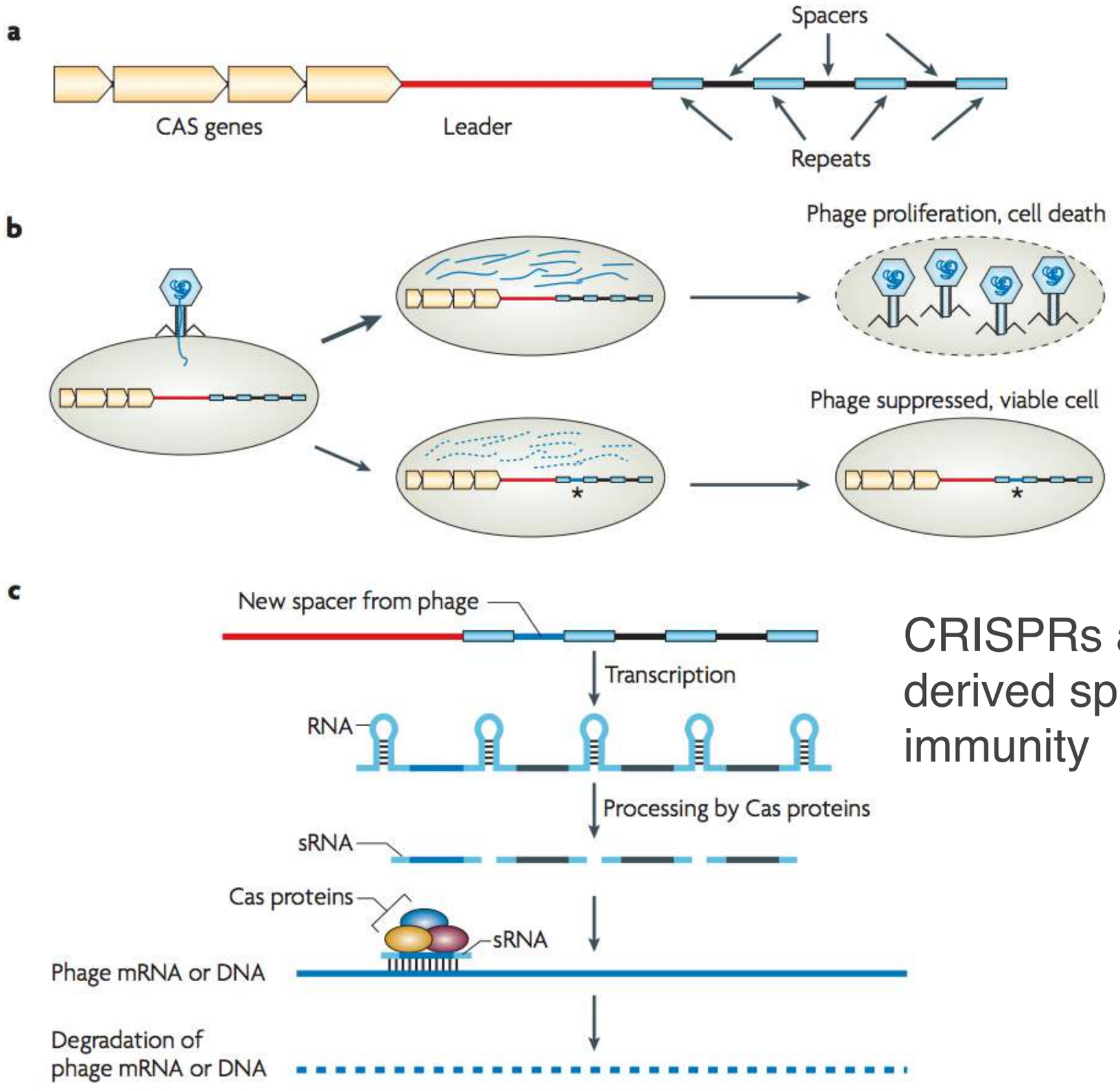
CRISPR–Cas systems identified in uncultivated organisms

Only 1% of the microbes are cultivable



Burstein et al., 2017

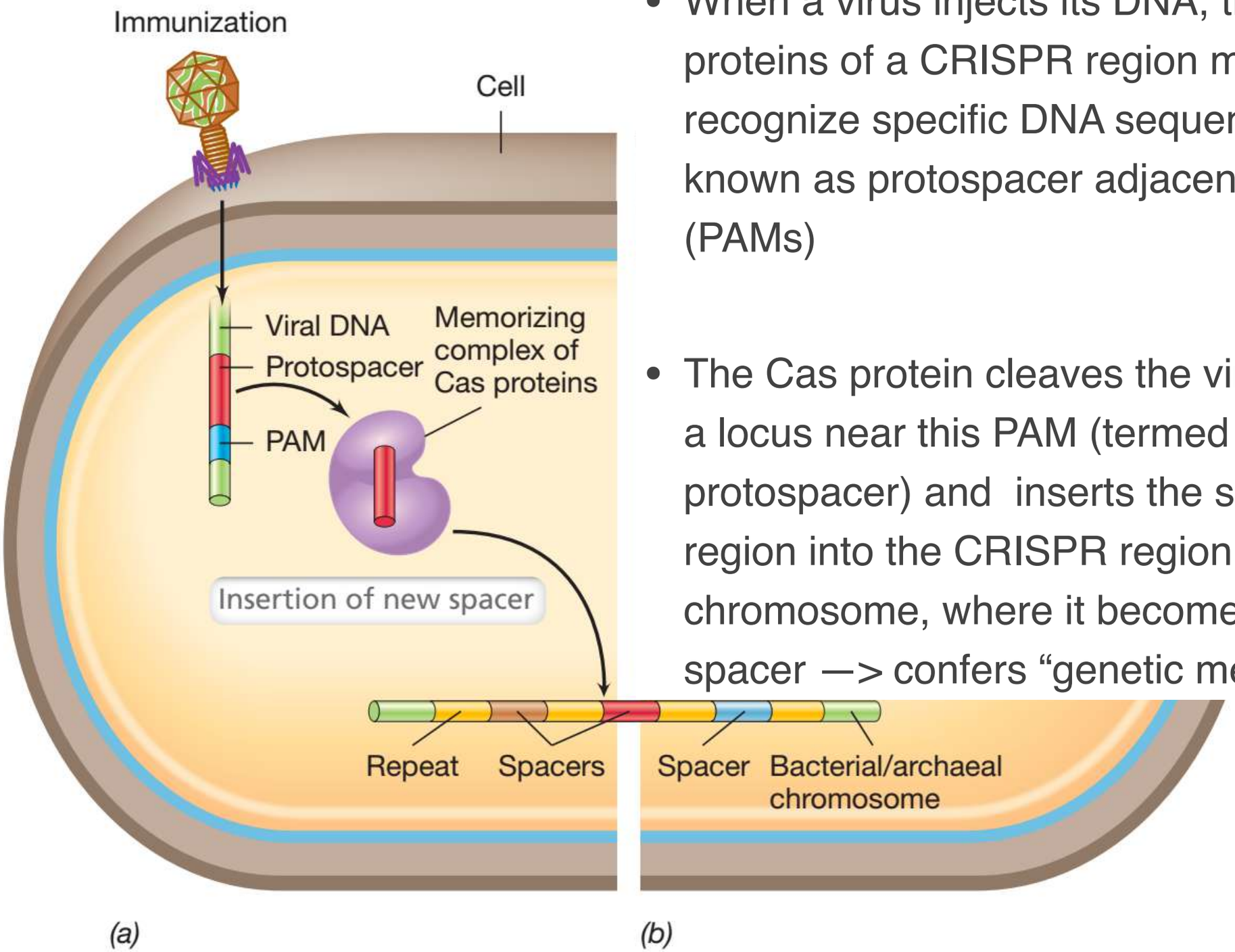
Simplified model for CRISPR action



CRISPRs acquire phage-derived spacers that provide immunity

Sorek et al., 2008

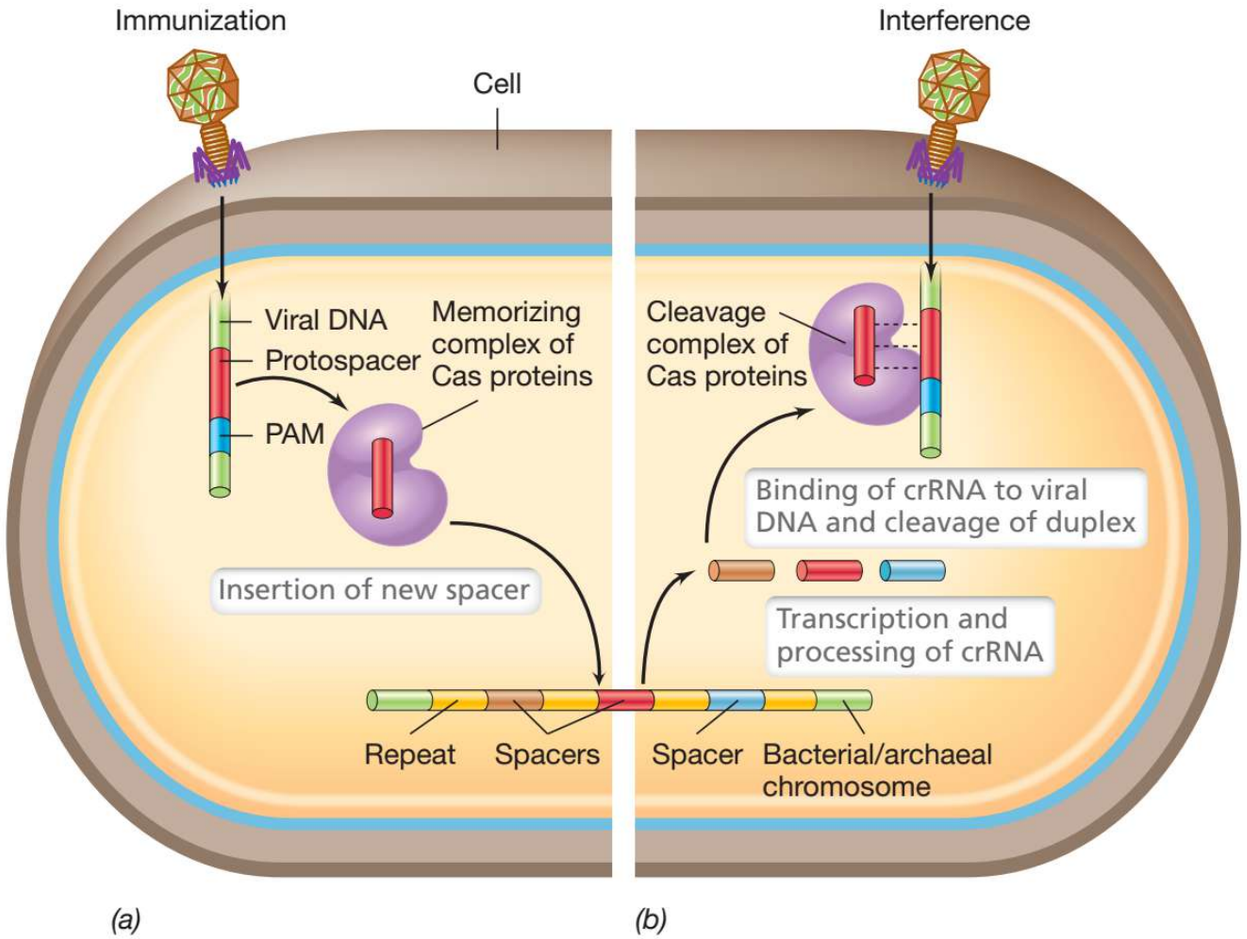
CRISPR, the clustered regularly interspaced short palindromic repeats, II



Madigan et al. 2018

- When a virus injects its DNA, the Cas proteins of a CRISPR region may recognize specific DNA sequences known as protospacer adjacent motifs (PAMs)
- The Cas protein cleaves the viral DNA at a locus near this PAM (termed the protospacer) and inserts the short DNA region into the CRISPR region of the chromosome, where it becomes a spacer —> confers “genetic memory”

CRISPR, the clustered regularly interspaced short palindromic repeats, III

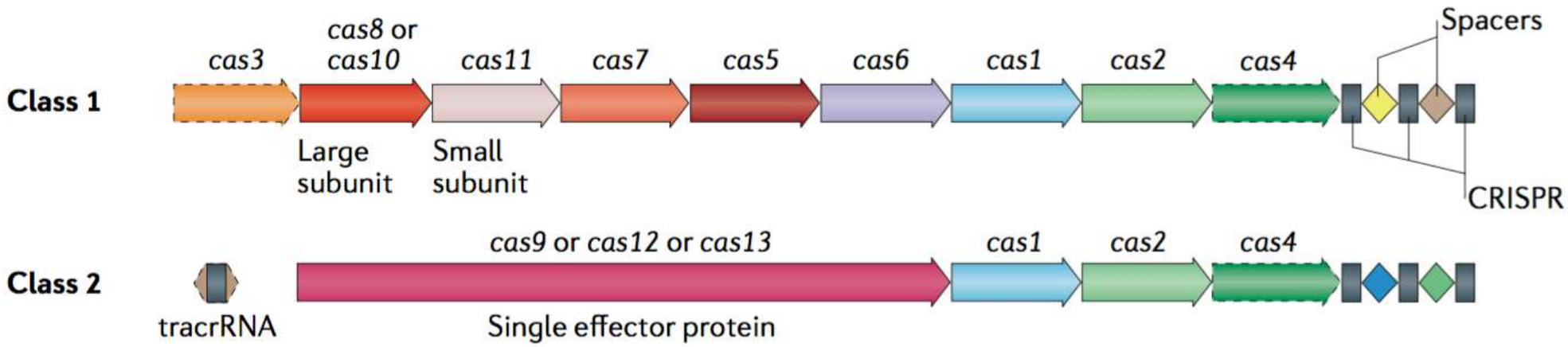


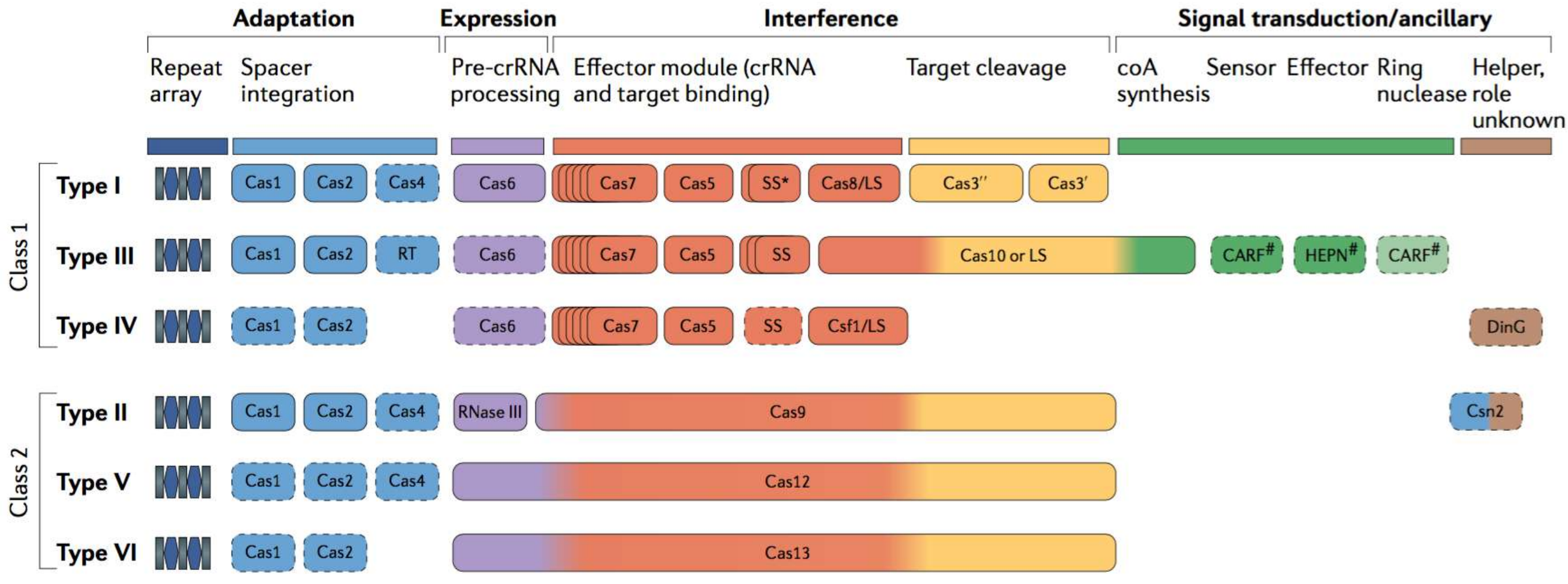
Madigan et al. 2020

- **Transcription** of **Pre-CRISPR RNA** (pre-crRNA) contains an array of RNA sequences **complementary to repeat and spacer regions**
- Cas proteins process the transcript into individual spacer RNAs by targeting the repeat regions
- **crRNAs+Cas surveillance for complementary incoming viral DNAs**

- **Any viral DNA:crRNA duplexes formed are cleaved by Cas endonuclease activity**
- Invading **DNA is degraded** in a process called ***interference***
- With part of its **genome destroyed** an invading virus **cannot proceed to replicate**
- **Immunization** when virus has been **inactivated by environmental factors** (e.g. UV radiation) or when the host's **restriction enzyme system cleaves** the invading DNA prior infection's begin

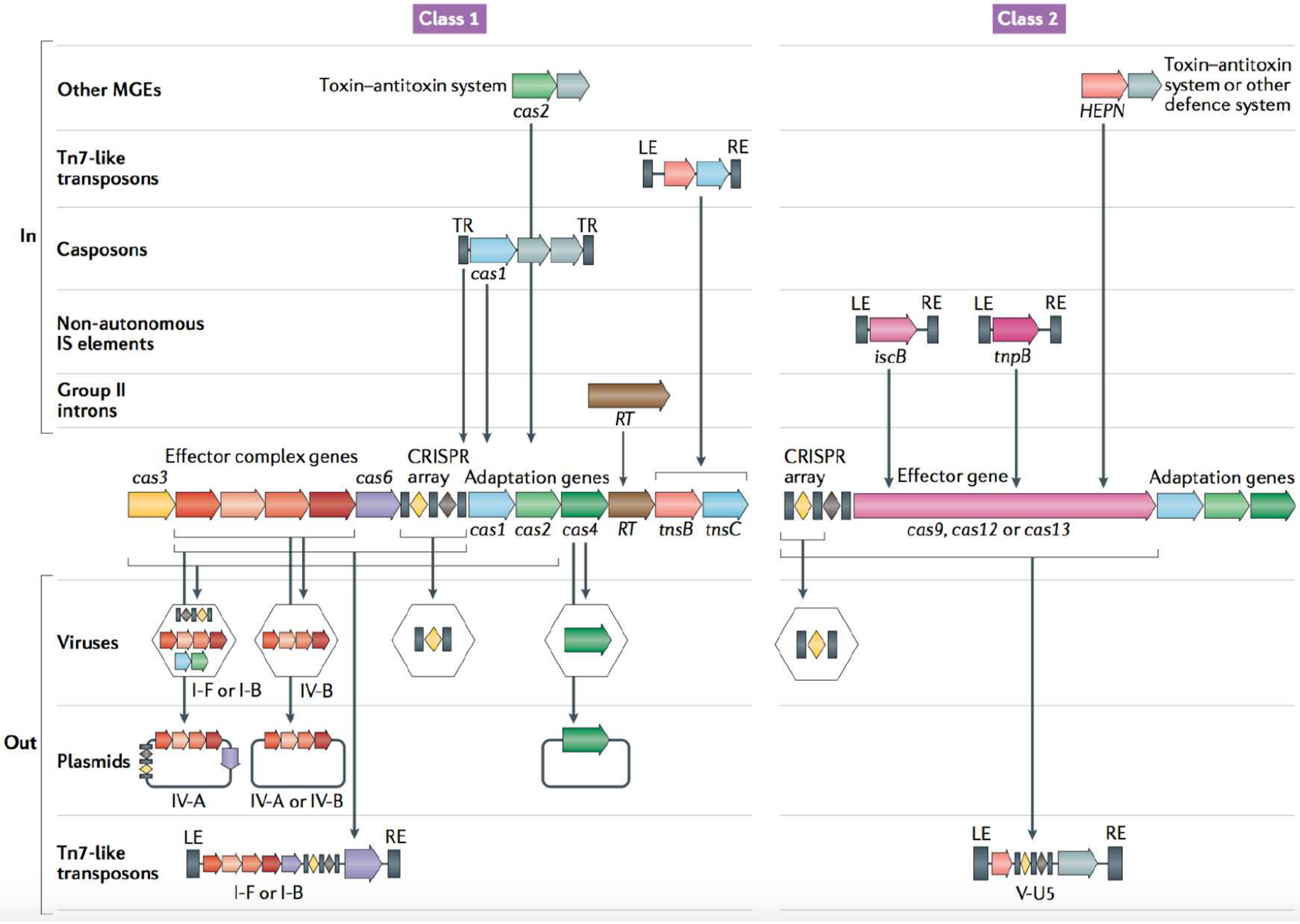
2 CRISPR classes and 6 types





Makarova et al., 2020

Evolution within the continuum



Faure et al., 2019

Distribution of the six types of CRISPR–Cas system in the major archaeal and bacterial phyla

Makarova et al., 2020

CRISPR–*cas*
Archaeal and bacterial system of adaptive immunity that consists of a CRISPR array and *cas* genes.

pre-crRNA
Long transcript of a CRISPR locus that is processed to yield the crRNA CRISPR–Cas system, where it is incorporated as a spacer.

crRNAs
Short RNA molecules containing the spacer sequence and parts of the CRISPR, used as the guide to target and cleave cognate foreign DNA or RNA.

CRISPR adaptation module
A group of ***cas*** genes dedicated to the selection and insertion of new spacers into CRISPR arrays.

Transposon
A mobile genetic element, typically flanked by inverted terminal repeats, that changes its location in the host genome by inserting into new sites with the help of a transposon-encoded enzyme known as transposase, integrase or recombinase.

CRISPR effector module
A suite of Cas proteins (Class 1 CRISPR–Cas systems) or a single large protein (Class 2 CRISPR–Cas systems) that are responsible for maturation of the CRISPR RNA and interference.

Protospacer
A piece of DNA, typically from a mobile genetic element genome, that is inserted into a CRISPR array by the CRISPR adaptation complex, to become a spacer.

Interference
Final stage of the CRISPR–Cas response, which involves recognition and cleavage of the target DNA or RNA.

Protospacer-adjacent motif
(PAM). A short nucleotide sequence next to the protospacer that is required for target recognition by the crRNA effector.

Protospacer
Segment of DNA (typically, from a virus or plasmid) that is acquired by CRISPR–Cas systems via the activity of the adaptation complex.

CRISPR array
Genomic locus containing multiple, tandem CRISPR.

Spacer
Unique segment of DNA inserted between CRISPR units.

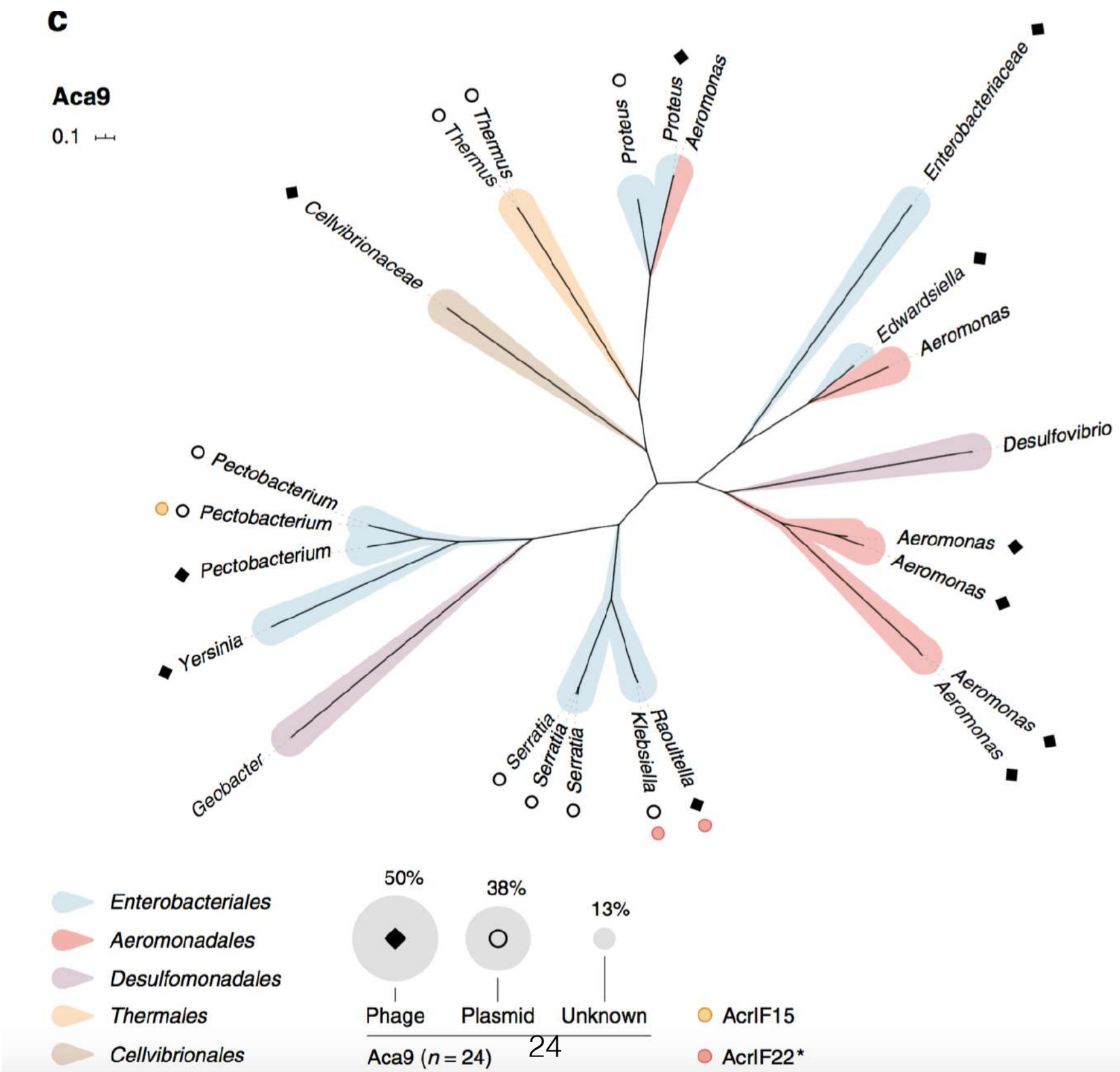
1. Borges, A. L., Davidson, A. R. & Bondy-Denomy, J. The discovery, mechanisms, and evolutionary impact of anti-CRISPRs. Annu Rev. Virol. 4, 37–59 (2018).

2. Bondy-Denomy Pawluk, A., Maxwell, K. L. & Davidson, A. R. Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system. Nature 493, 429–432 (2013).

Multiple anti-CRISPRs

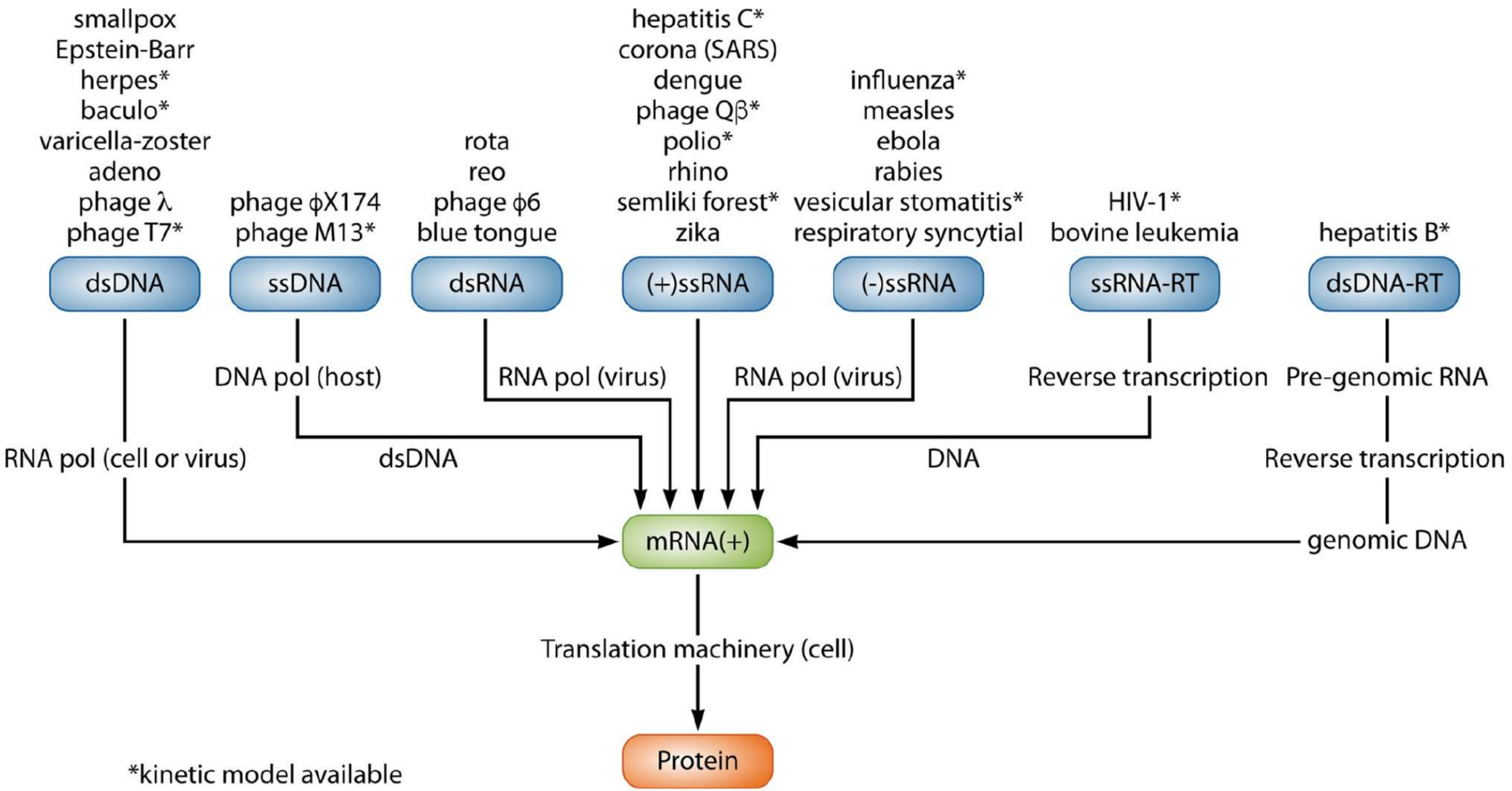
- Over evolution time scale, at the microbial level the war continues to act and react to invaders
- Mobile Genetic Elements (MGEs) have developed inhibitors of CRISPR–Cas function called anti-CRISPR (Acr) proteins
- The first acr genes were discovered in phages that inhibit the type I–F CRISPR–Cas system of *Pseudomonas aeruginosa*
- Acr proteins has revealed a diverse range of inhibitory activities, including interference with crRNA loading, inhibition of target DNA recognition, and inhibition of DNA cleavage

Phylogenetic diversity of Aca9



Pinilla-Redondo et al., 2020

Hijacking host metabolism



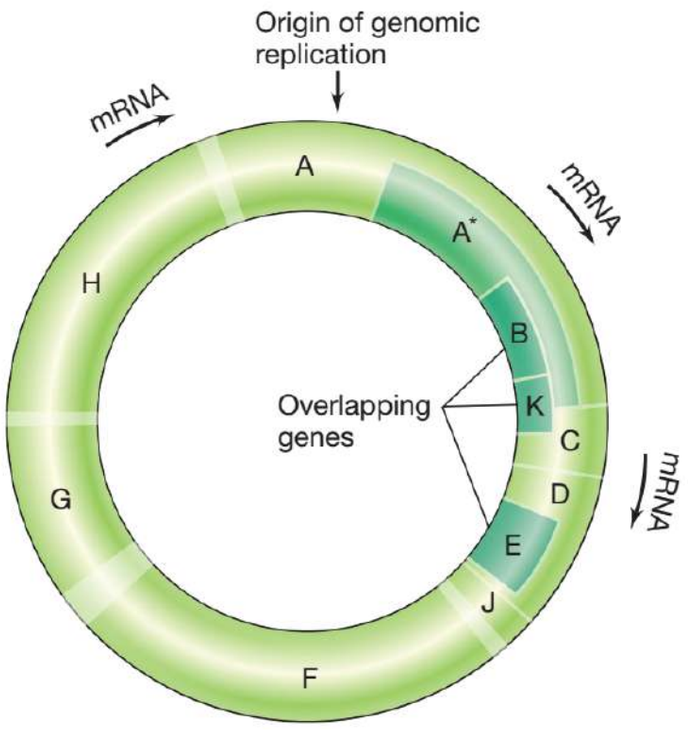
Yin & Redovich, 2018

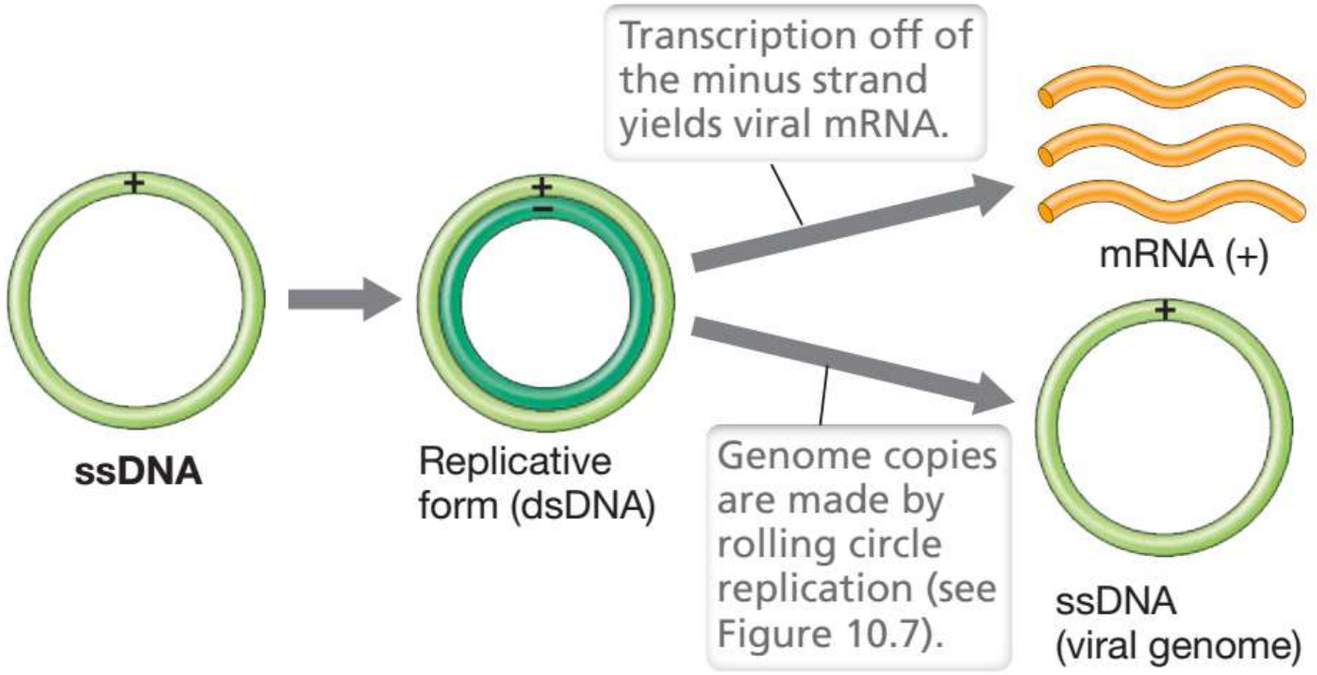
Bacteriophage fX174 (*E. coli* host)

- Bacteriophage fX174, ssDNA —> **overlapping genes**, a condition in which there is **insufficient DNA** to encode all viral-specific proteins unless **parts of the genome are read more than once in different reading frames**

- The distinct gene products from overlapping genes are made by **reinitiating transcription in a different reading frame** within a gene to yield a second (and distinct) transcript

Madigan et al. 2020

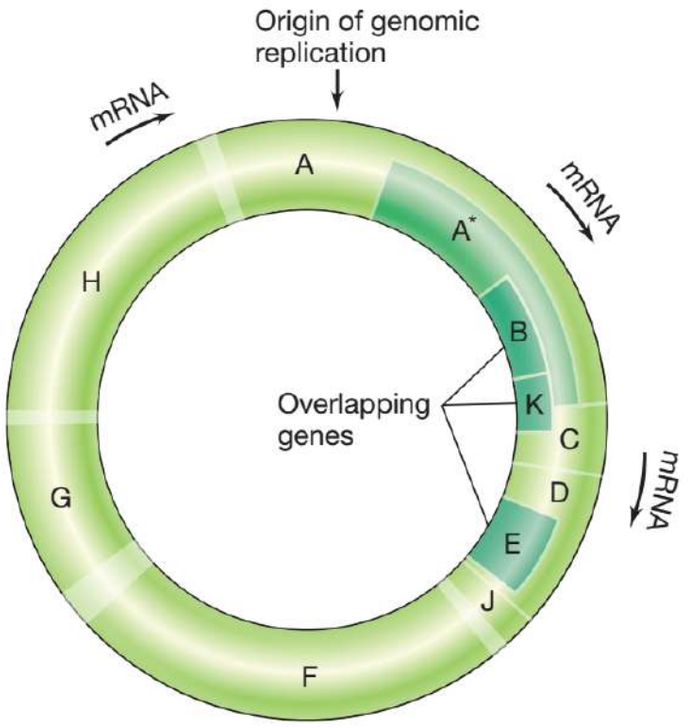




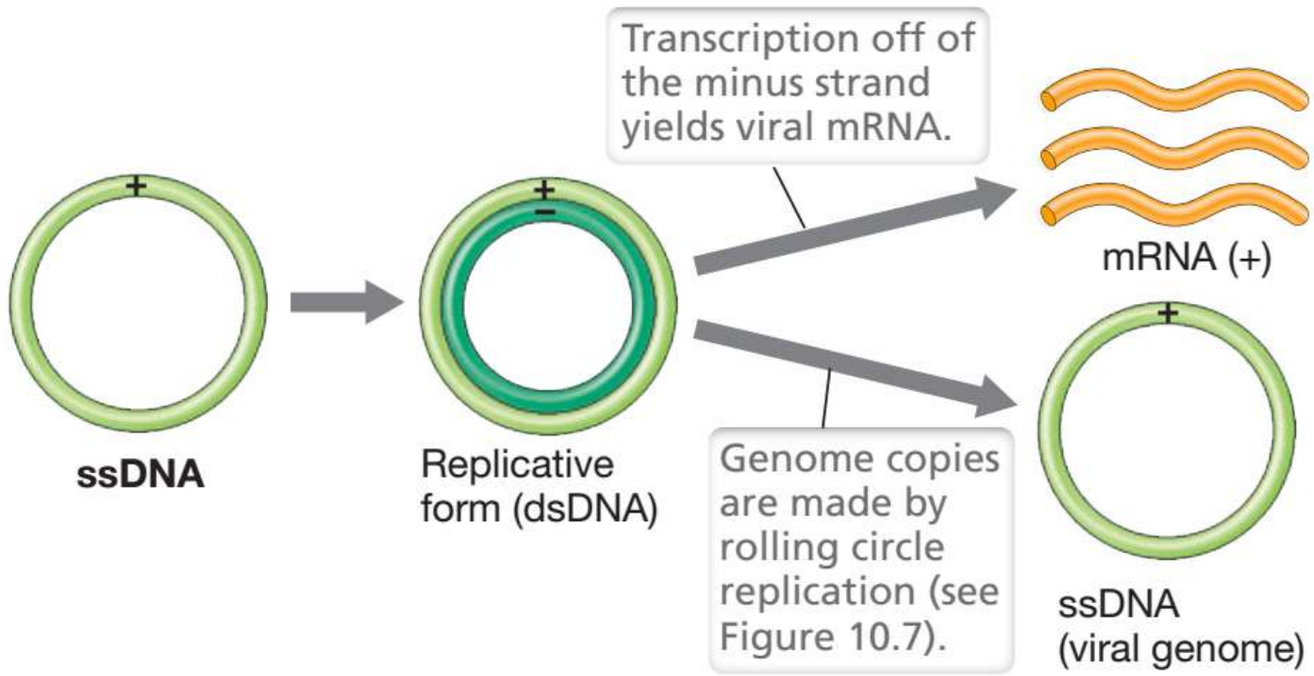
(b) **Flow of events during ϕX174 replication**

Bacteriophage fX174 (*E. coli* host)

- Before a single-stranded DNA genome (ssDNA +) can be transcribed, a complementary strand of DNA must be synthesized, **forming a double-stranded molecule called the replicative** form for producing both mRNA and genome copies



Madigan et al. 2020

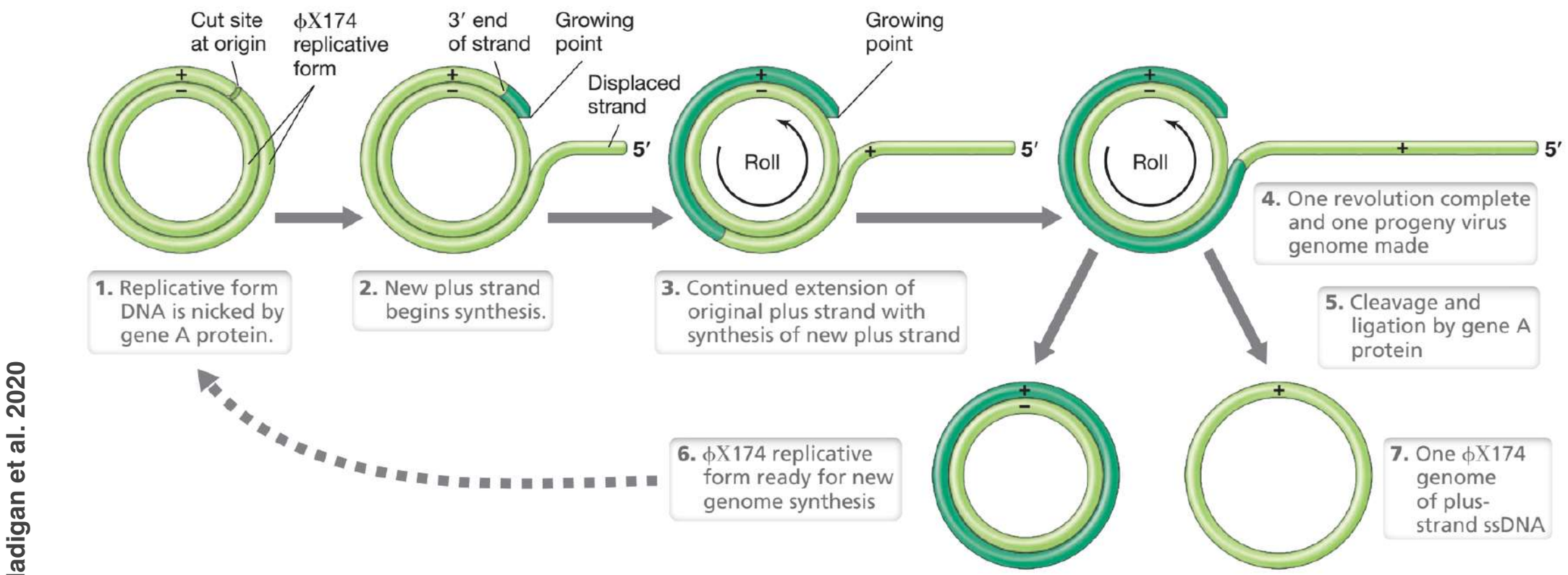


(b) **Flow of events during ϕX174 replication**

Bacteriophage fX174 (*E. coli* host)

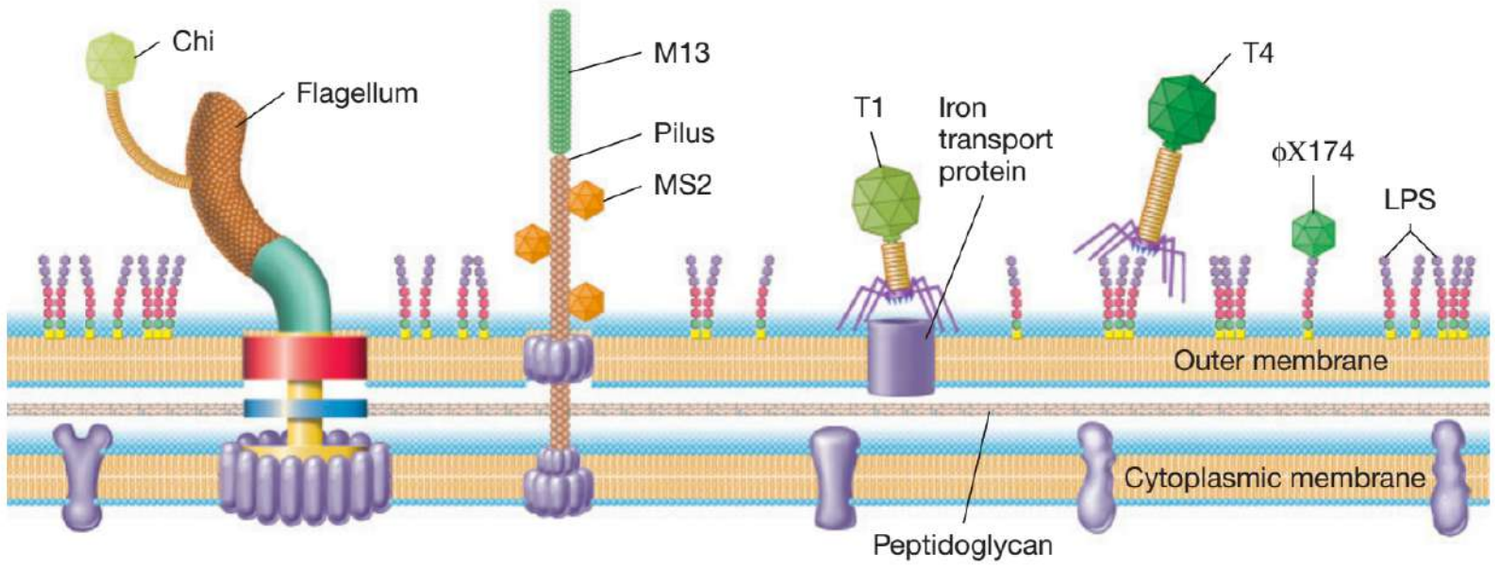
Rolling circle replication facilitates the **continuous production of positive strands** from the replicative form

Note that rolling circle synthesis differs from semiconservative replication because **only the negative strand serves as a template**



Madigan et al. 2020

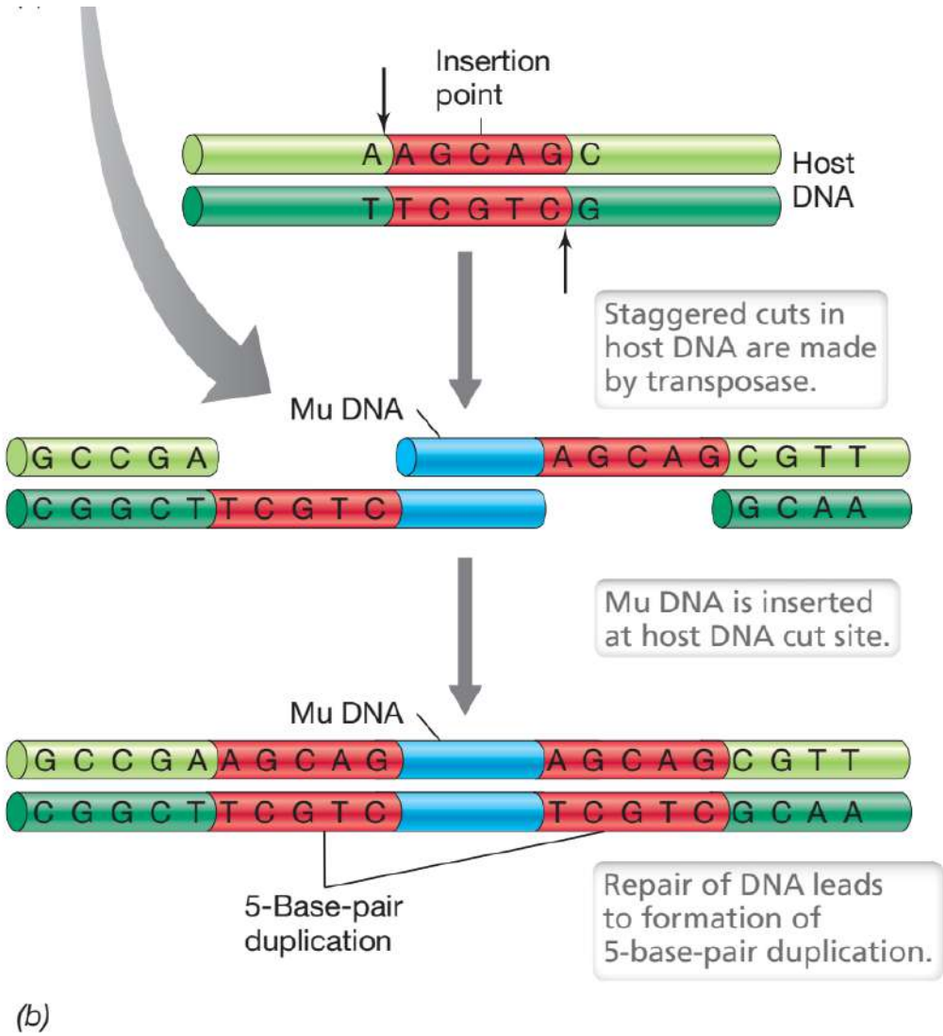
Bacteriophage M13 (*E. coli* host)



- M13, **ssDNA**, have the unusual property of being **released** from the host cell **without** the cell undergoing **lysis** infected cells continue to grow
- **Chronic infection**
- M13 DNA is **covered** with **coat proteins** as it exits across the cell
- **No intracellular accumulation** of mature virions as in typical **lytic cycle**
- A double-stranded form of genomic DNA essential for cloning purposes is produced naturally when M13 produces its **replicative form**

Bacteriophage Mu (*E. coli* host)

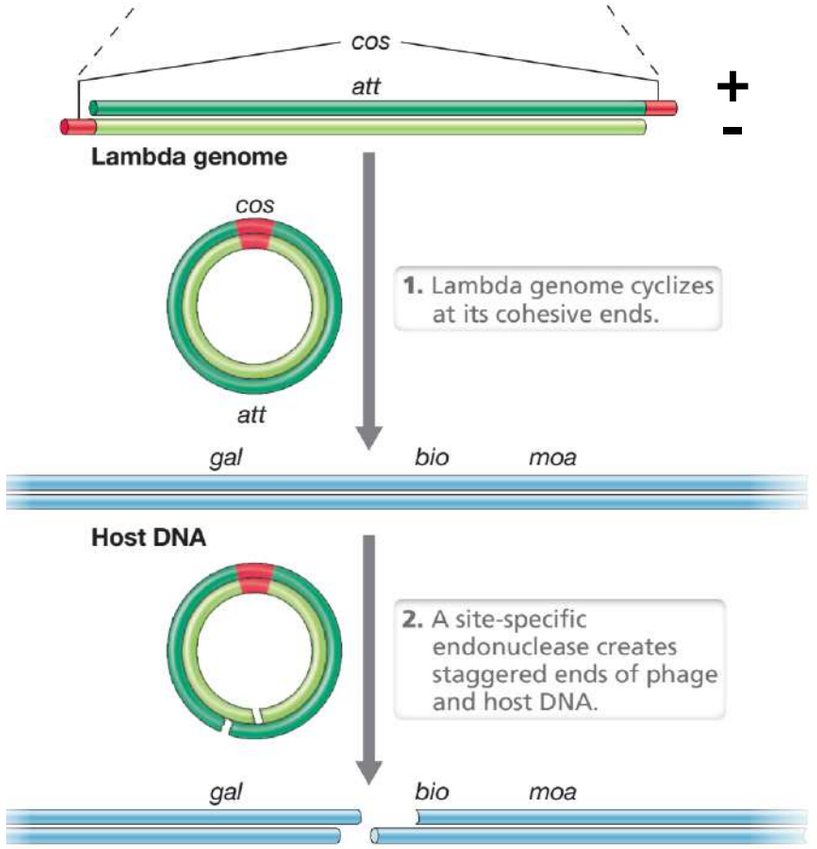
- Bacteriophage **Mu, ds DNA is a temperate phage**
- **Replicating by transposition**
- Transposable elements are **sequences of DNA** that can **move within their host genome** from one location to another as discrete genetic units
- Transposition is facilitated by **transposase**
- **Mu** is named because it **generates mutations** when it integrates into the host cell chromosome
- Integration of Mu DNA into host genome
- Integration requires the activity of Mu **transposes and a 5-base-pair fragment of host DNA is duplicated** at the target site where Mu DNA is integrated
- This host DNA duplication arises because staggered cuts are made at the point in the host genome where Mu DNA is inserted

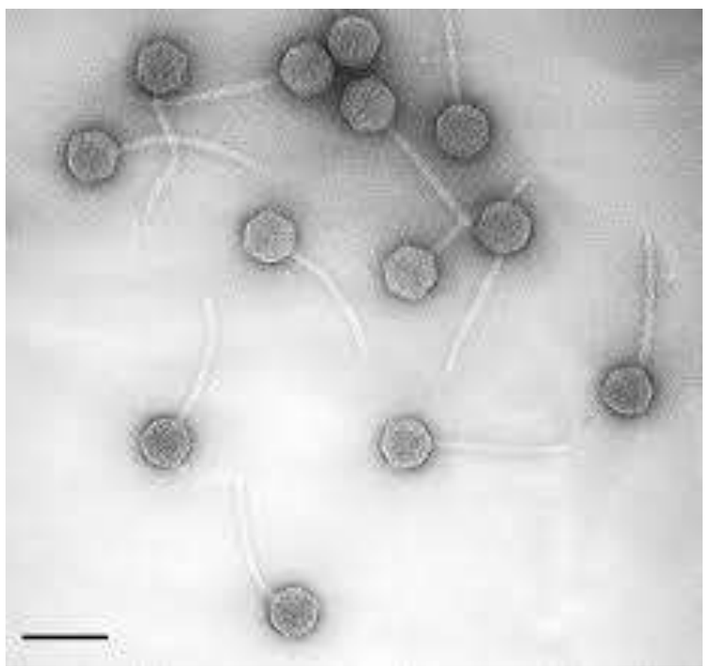


Madigan et al. 2018

Bacteriophage lambda (*E. coli*, host)

- ds DNA —> *E. coli*
- **Lysogenic cycle**, due to host DNA damage
- **Lambda integrase** produced by phage
- Integrase **recognizes sites on phage and host genomes (att)**
- **att** within in the two cohesive ends (**cos**)
- Endonuclease cuts are different (**staggered ends**) on + and - strands

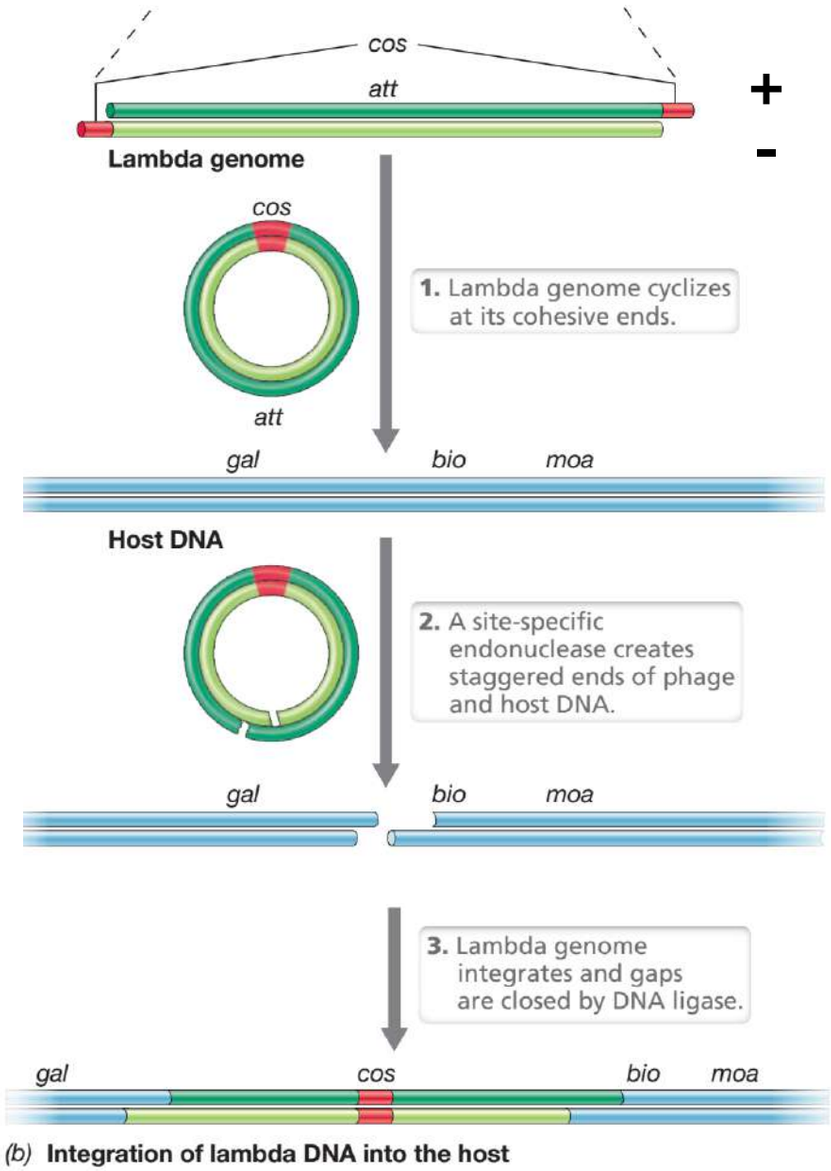




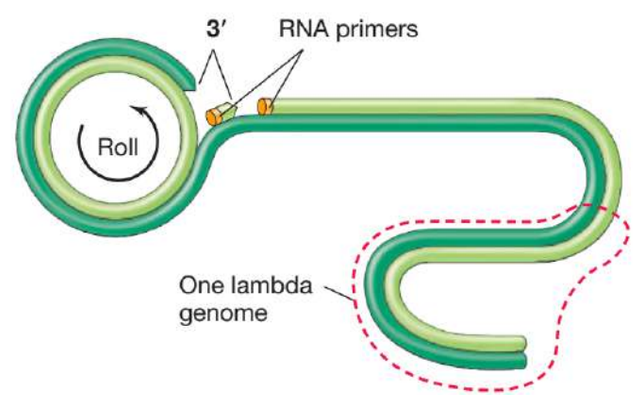
Madigan et al. 2020

Integration of viral DNA and rolling circle replication

- At 5’ is a single stranded region 12 nucleotides long- “**cohesive**” ends are complementary in base sequence (**cos**)
- When lambda DNA enters host cell: from **linear** to **circle** genome at the **cos** sites
- **Lytic** pathway, long, linear concatemers of genomic DNA by **rolling circle** replication
- **One strand in circular lambda genome is nicked and is “rolled out”** as a **template** for synthesis of the complementary strand
- **Cut** of concatamer at **cos** sites on double strands



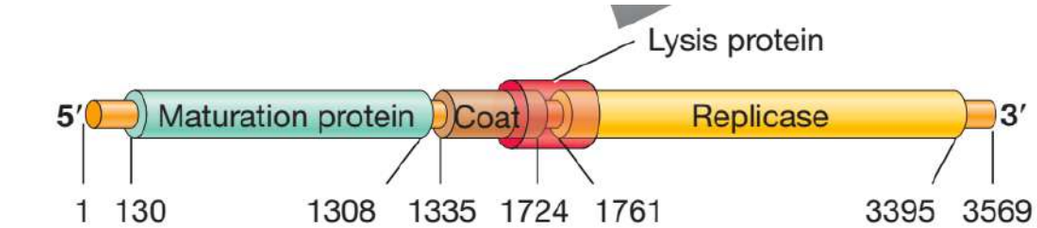
(b) **Integration of lambda DNA into the host**



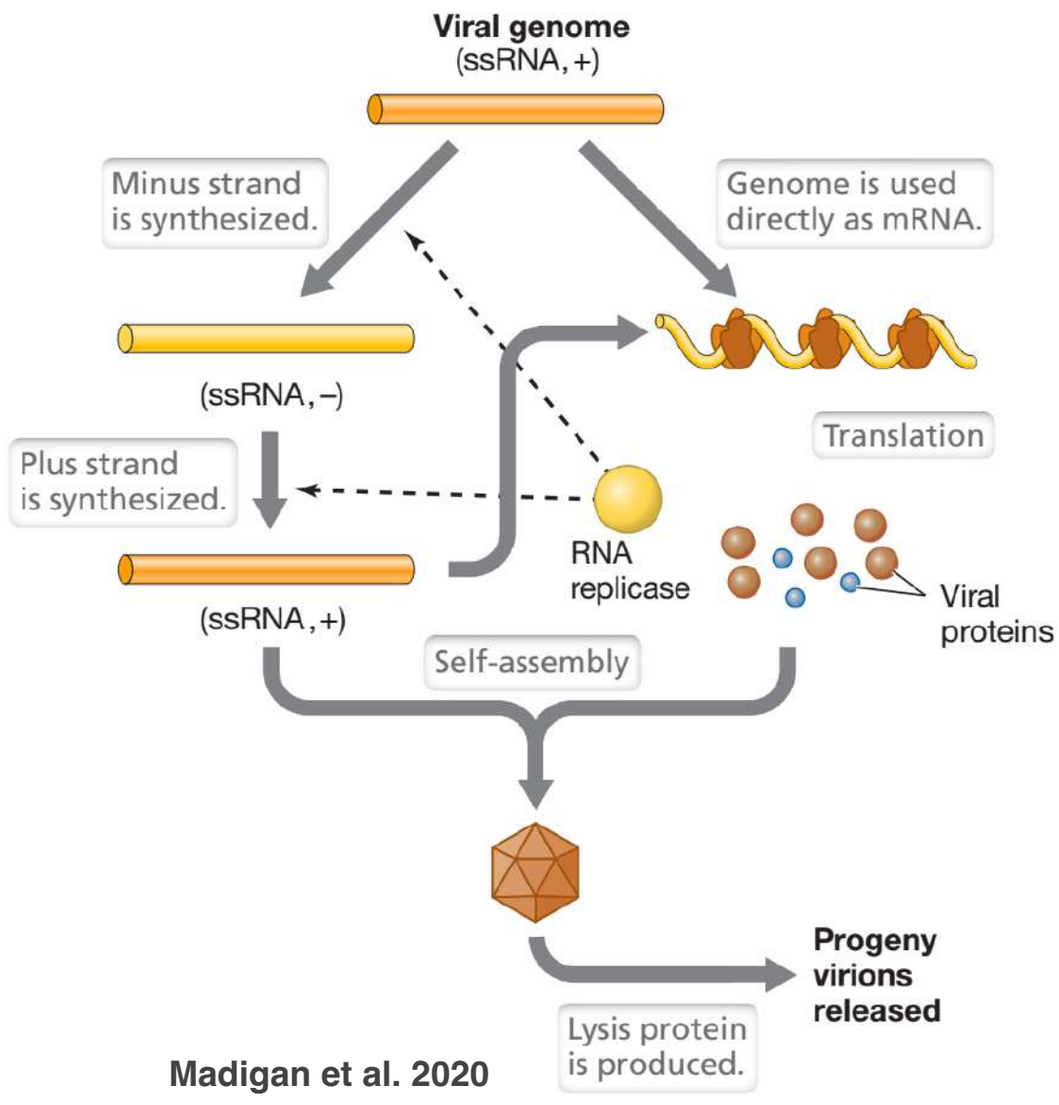
Madigan et al. 2020

Bacteriophage MS2 (*E. coli* host)

- **Positive ssRNA**
- **RNA replicase**, enzyme that replicates viral RNA
- **Host RNA polymerase** translate RNA+
- RNA replicase begins **synthesis of (-) RNA using (+)** strands as template
- RNA replicase begins **synthesis of (+) RNA using (-)** strands as template
- As (-) sense RNA copies accumulate, more (+)-sense RNA is made using the (-)-sense strands as templates —> **are translated for continued viral protein synthesis**



(b) **Genetic map of MS2 RNA genome**



Madigan et al. 2020

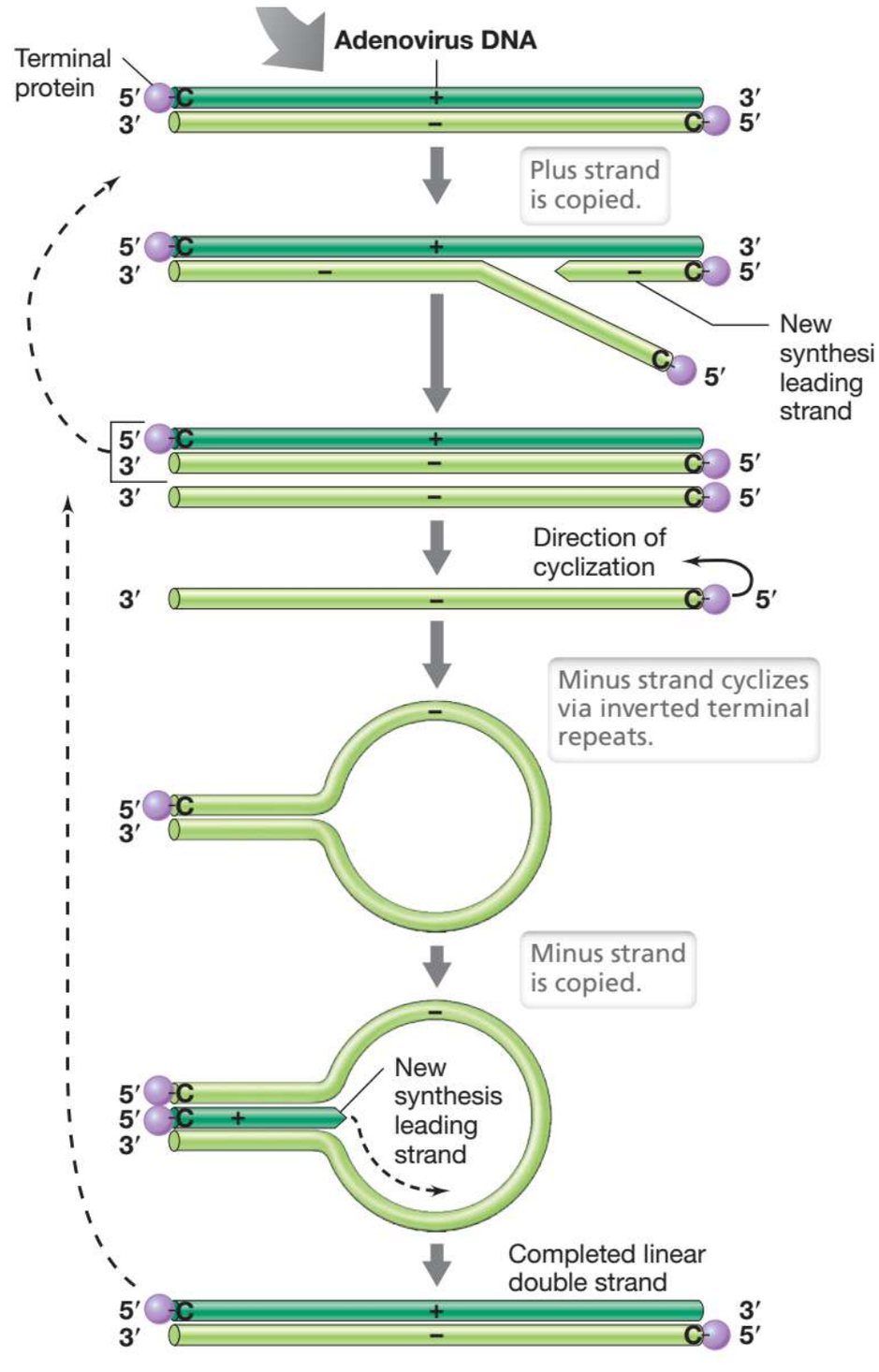
dsDNA (Euk)

Pox viruses—> all replication events, including DNA replication, occur in the **host cytoplasm** instead of the nucleus

Adenoviruses —> the replication of their genome proceeds in a **leading fashion on both DNA template strands**

The single minus DNA strand cyclizes by means of its inverted terminal repeats, and a complementary (plus-sense) DNA strand is synthesized beginning from its 5′ end

This mechanism is unique because double-stranded DNA is replicated **without** the formation of a **lagging strand,** as occurs in conventional semiconservative DNA replication

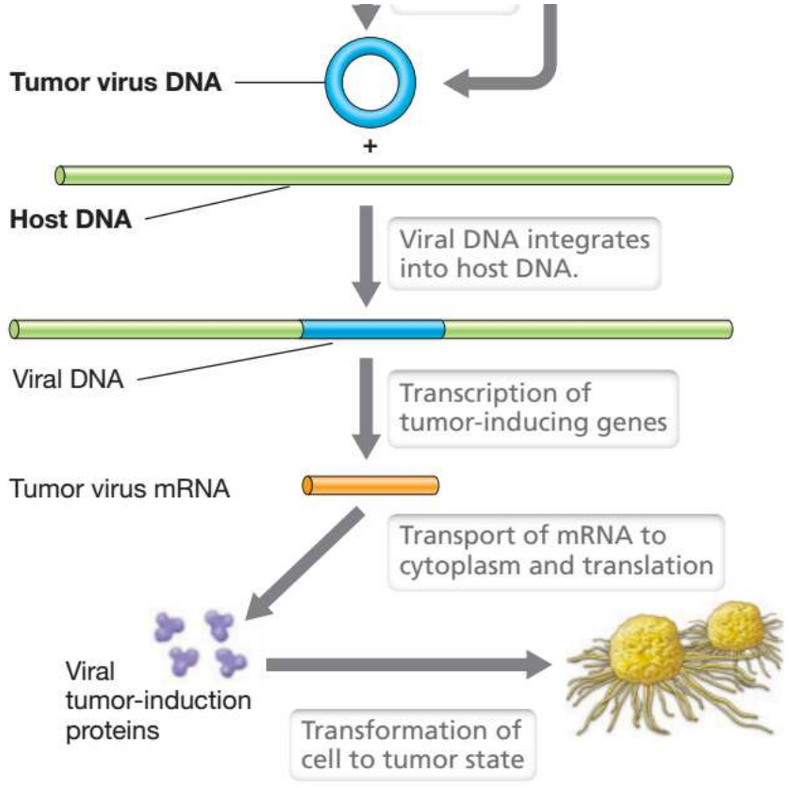


Madigan et al. 2018

dsDNA (Euk)

A. Polyomavirus SV40 small genome

- **If integrated** in host DNA **—> cancer**

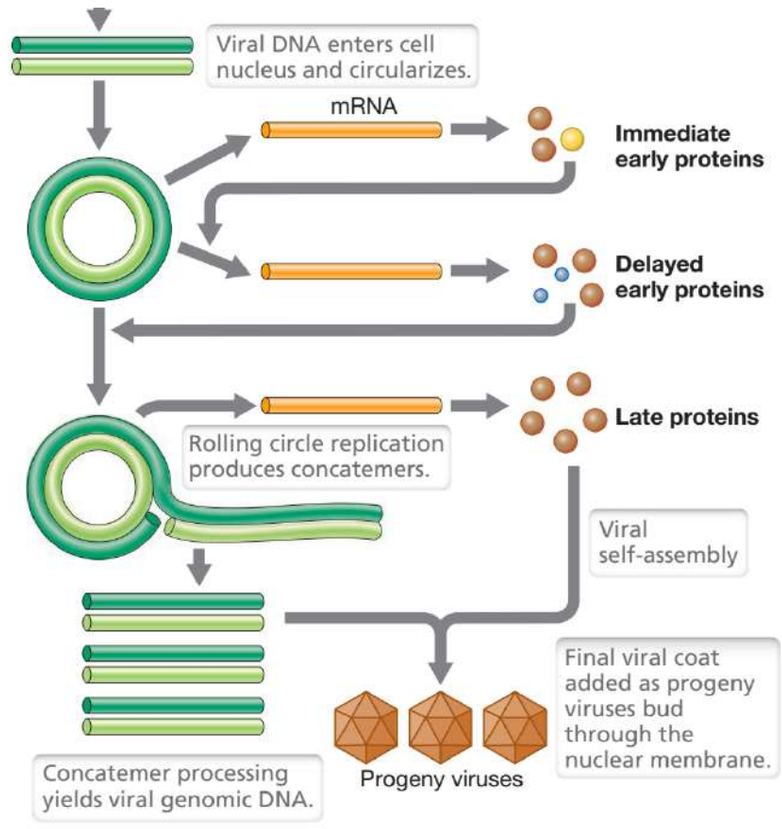


B. Herpesvirus different disease

- **Latent for long time**
- **Replication in the nucleus**

C. Cytomegalovirus (CMV) very common

- **For healthy individuals —> no symptoms**
- **CMV can cause pneumonia, retinitis certain gastrointestinal disorders**



Madigan et al. 2018