Structural Virology

Lecture 9

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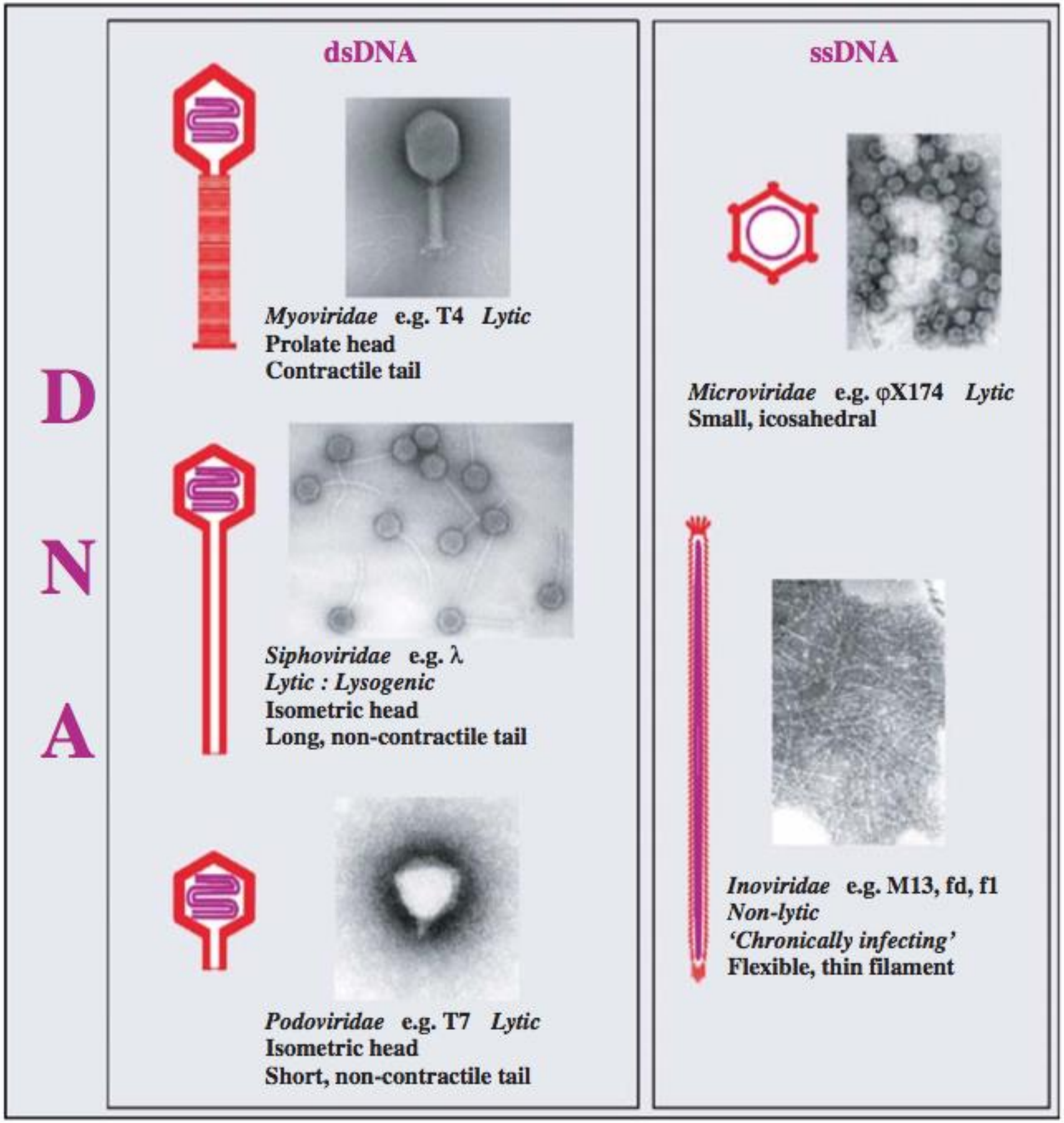
Financováno Evropskou unií
NextGenerationEU

NÁRODNÍ PLÁN OBNOVY

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

Bacteriophages



Myoviridae e.g. T4 *Lytic*
Prolate head
Contractile tail

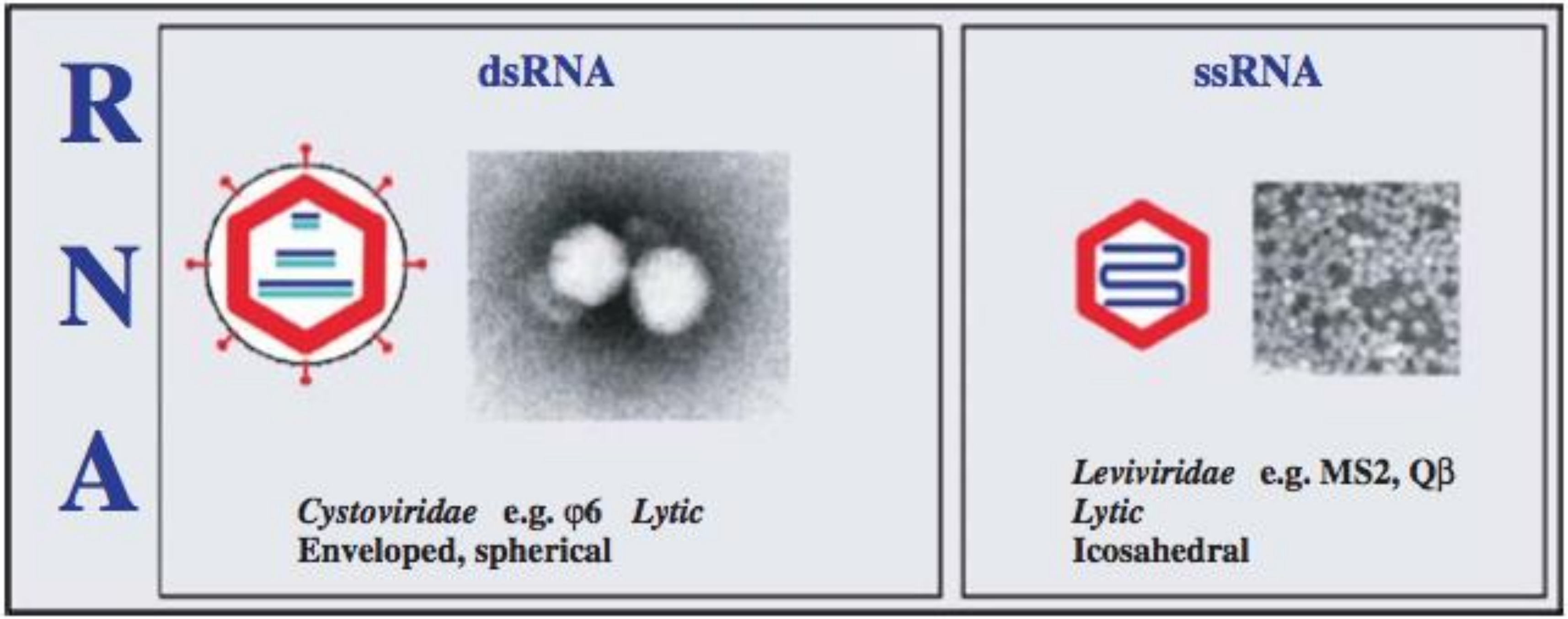
Siphoviridae e.g. λ
Lytic : Lysogenic
Isometric head
Long, non-contractile tail

Podoviridae e.g. T7 *Lytic*
Isometric head
Short, non-contractile tail

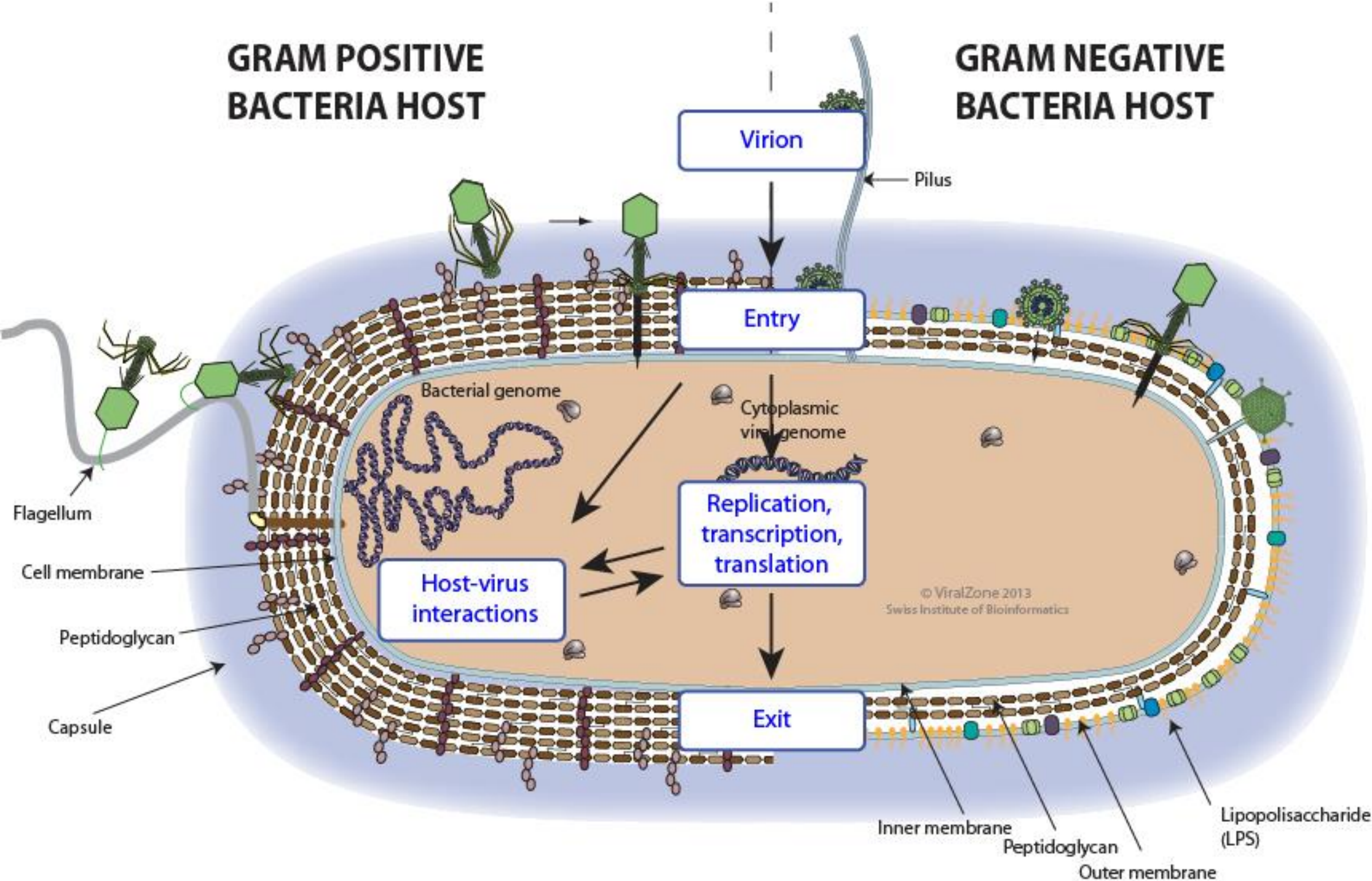
Microviridae e.g. φX174 *Lytic*
Small, icosahedral

Inoviridae e.g. M13, fd, f1
Non-lytic
'Chronically infecting'
Flexible, thin filament

Bacteriophages



Phages of gram positive and gram negative bacteria



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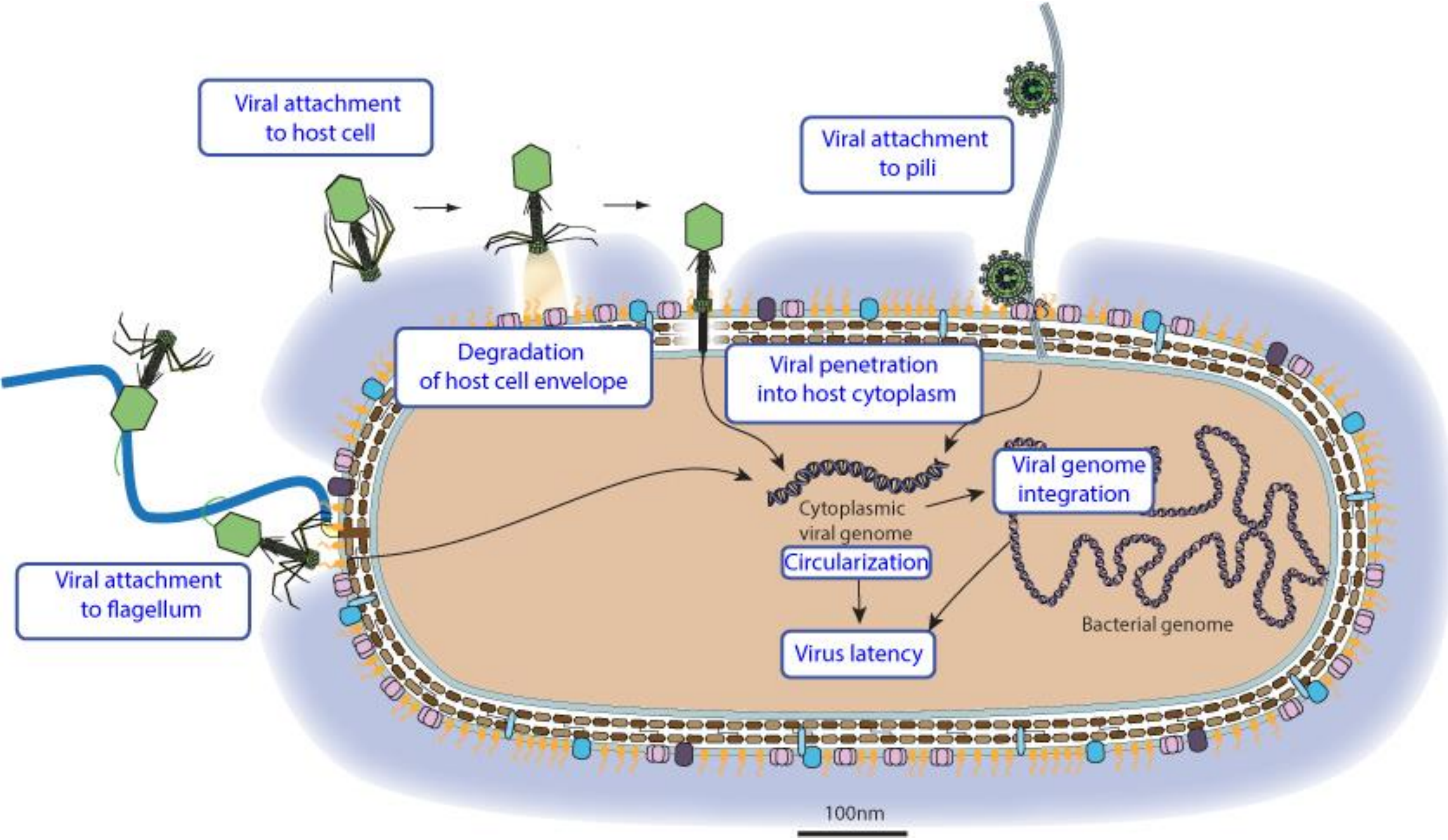
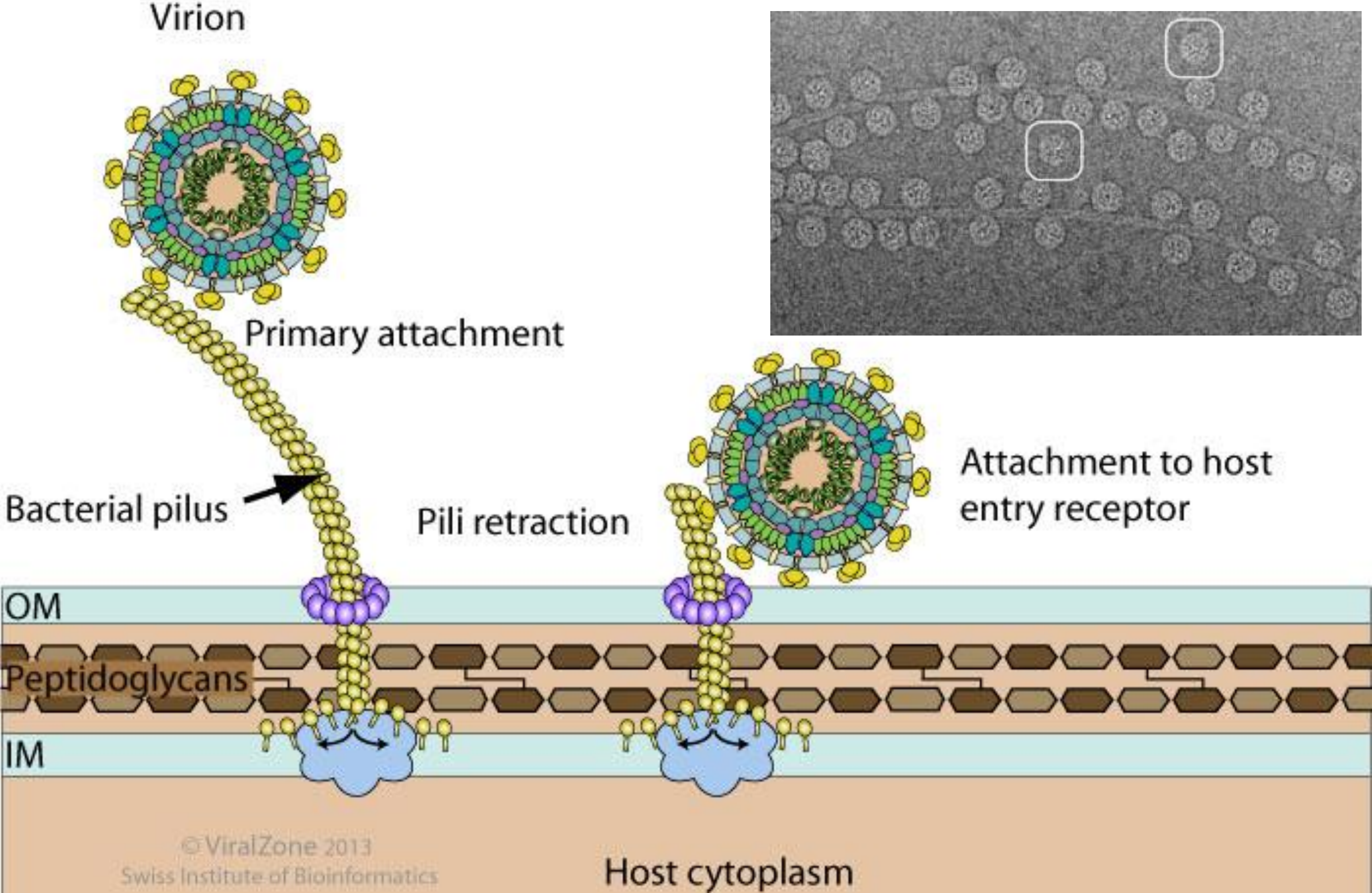
Phage entry


Viral attachment to host cell
Viral attachment to pili
Degradation of host cell envelope
Viral penetration into host cytoplasm
Viral genome integration
Cytoplasmic viral genome
Circularization
Virus latency
Bacterial genome
Viral attachment to flagellum
100nm

Viral attachment to host cell pilus



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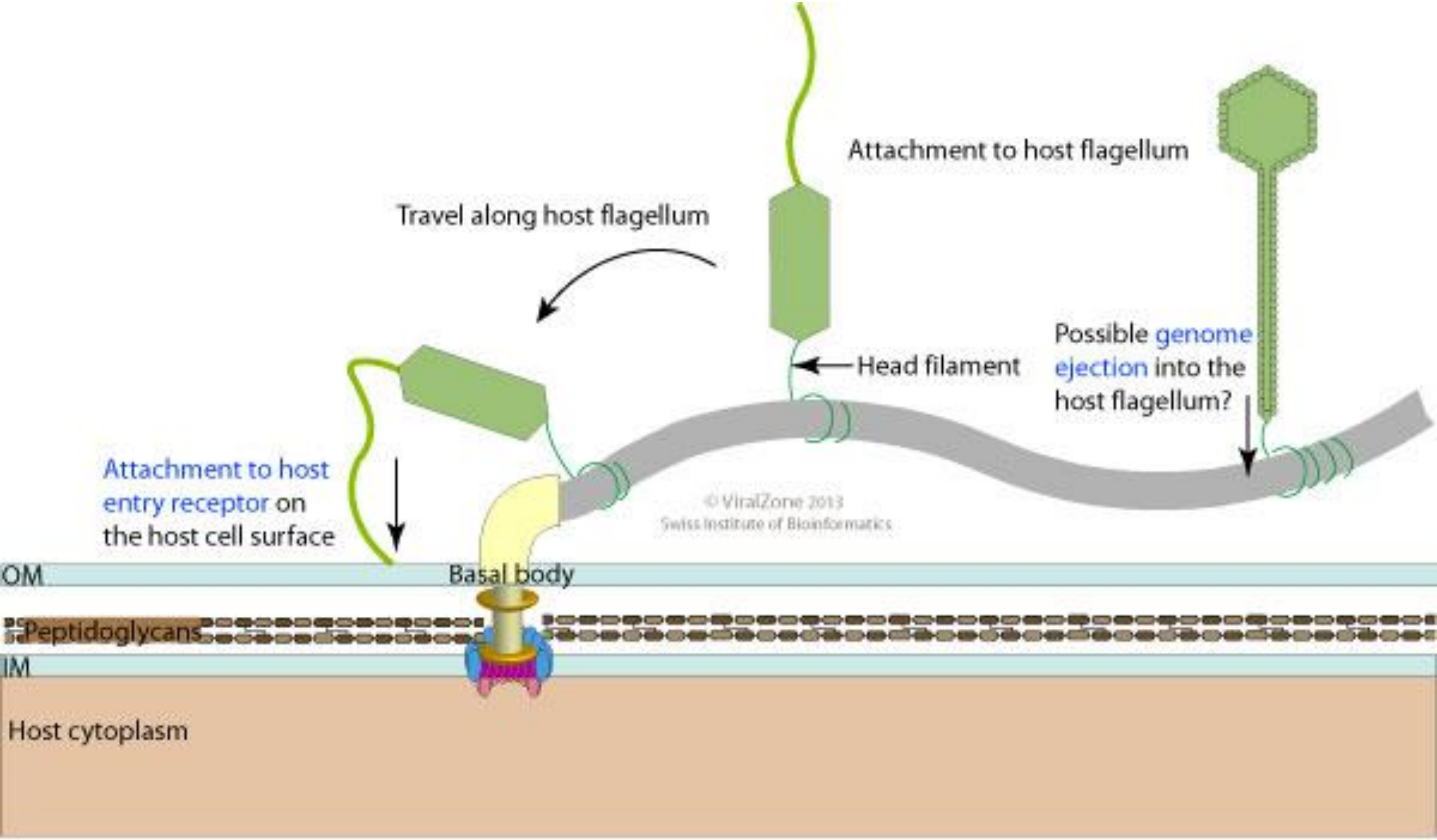
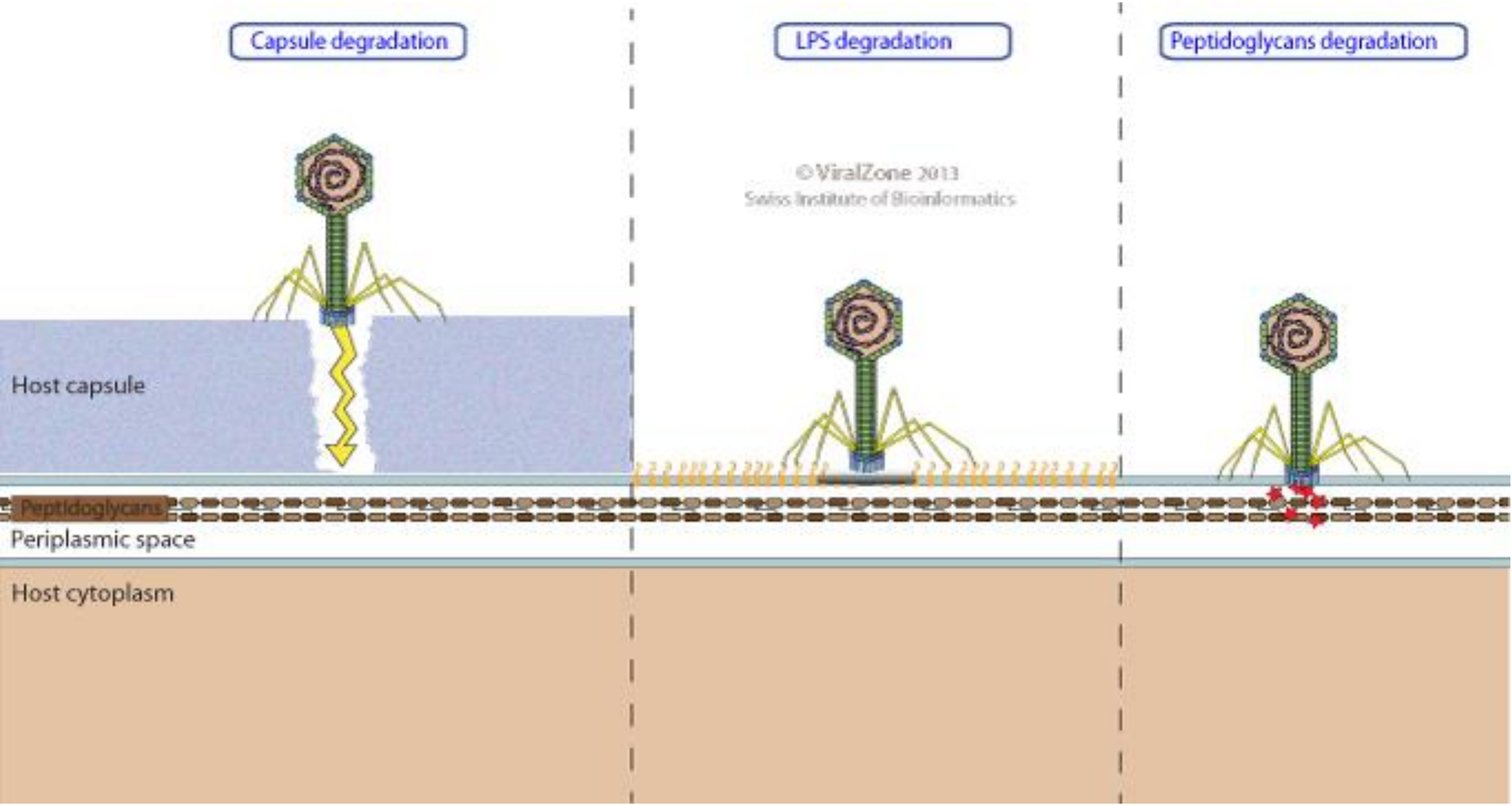
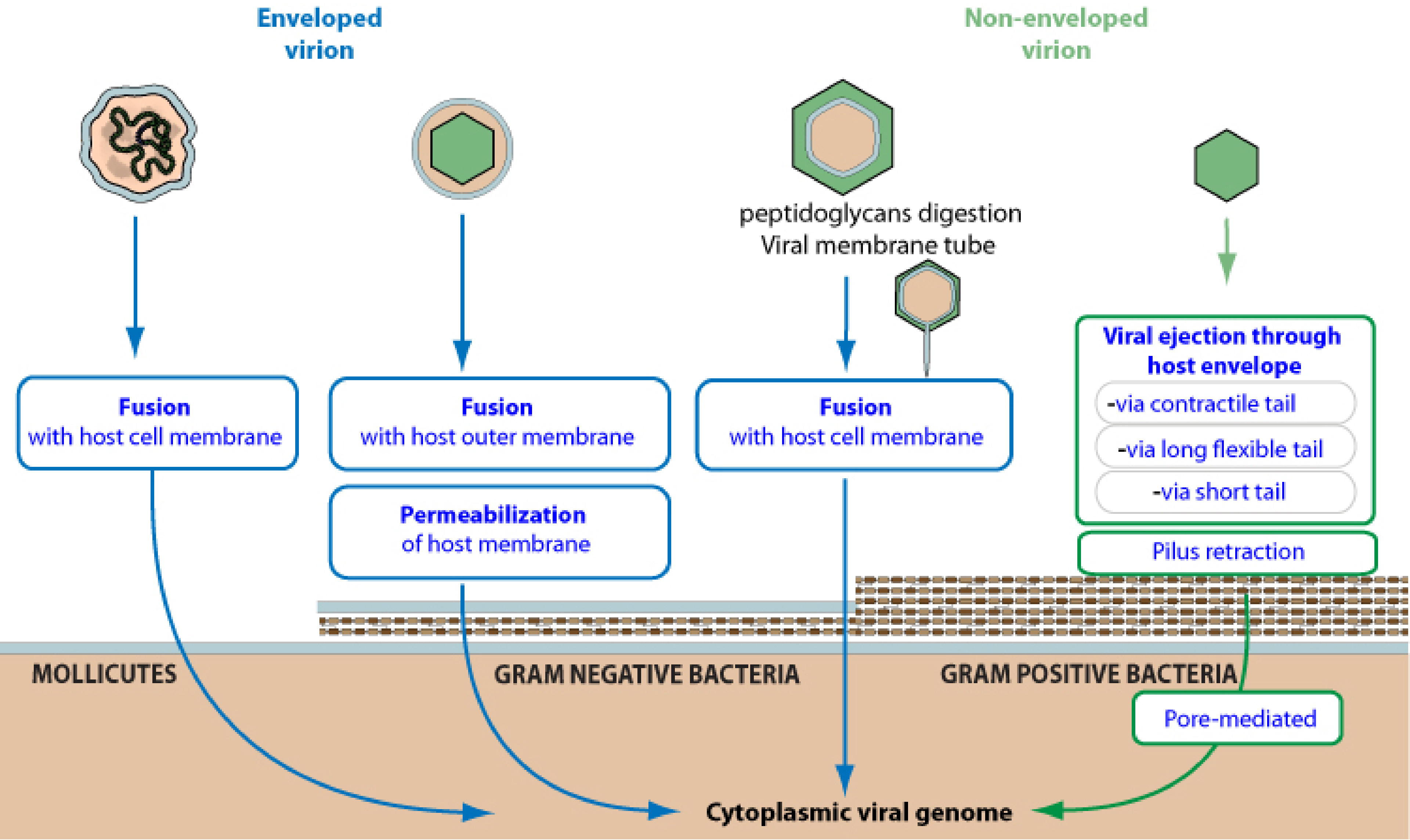
Viral attachment to host cell flagellum



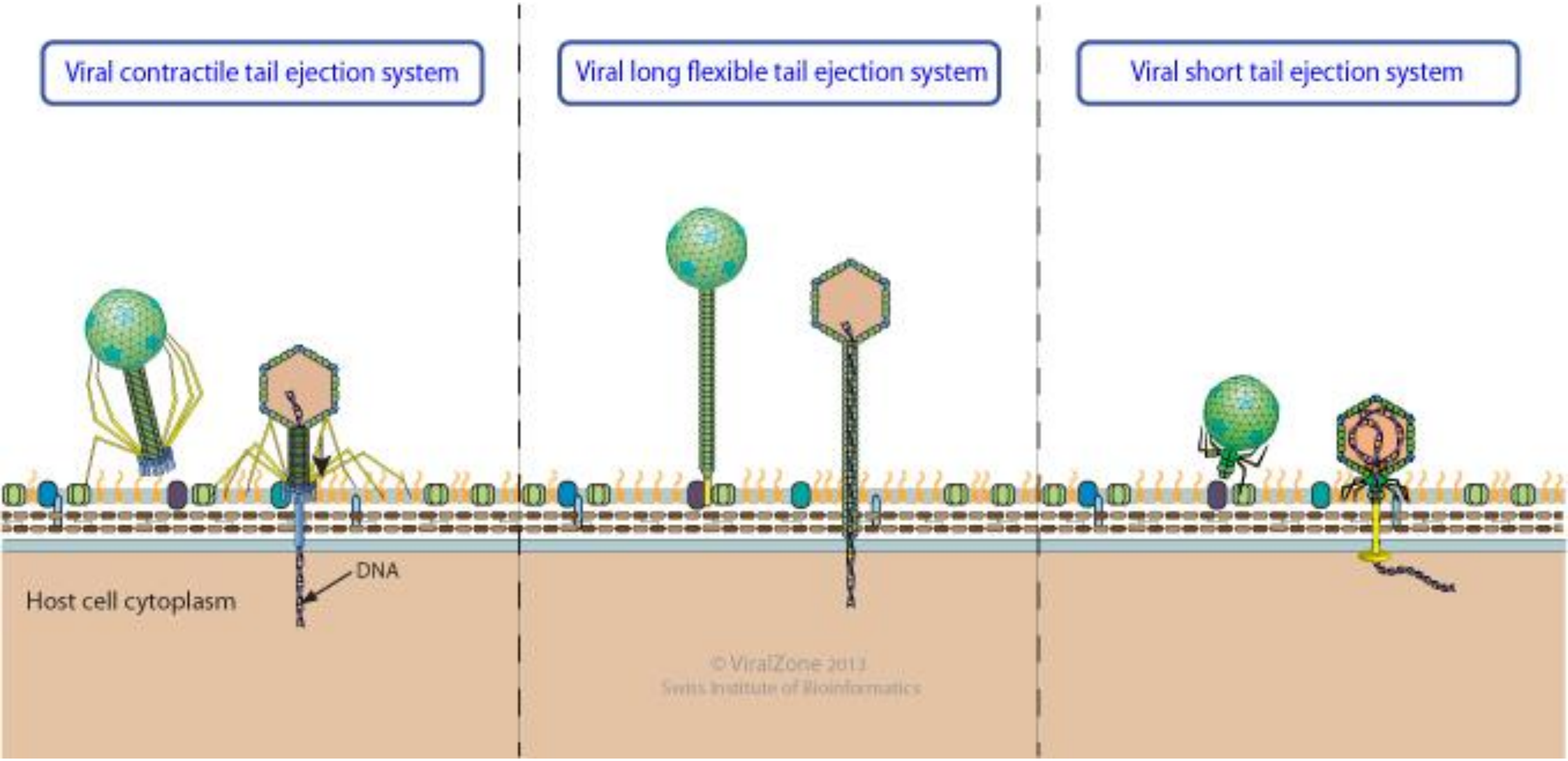
Degradation of host cell envelope components during virus entry



Phage penetration into host cytoplasm

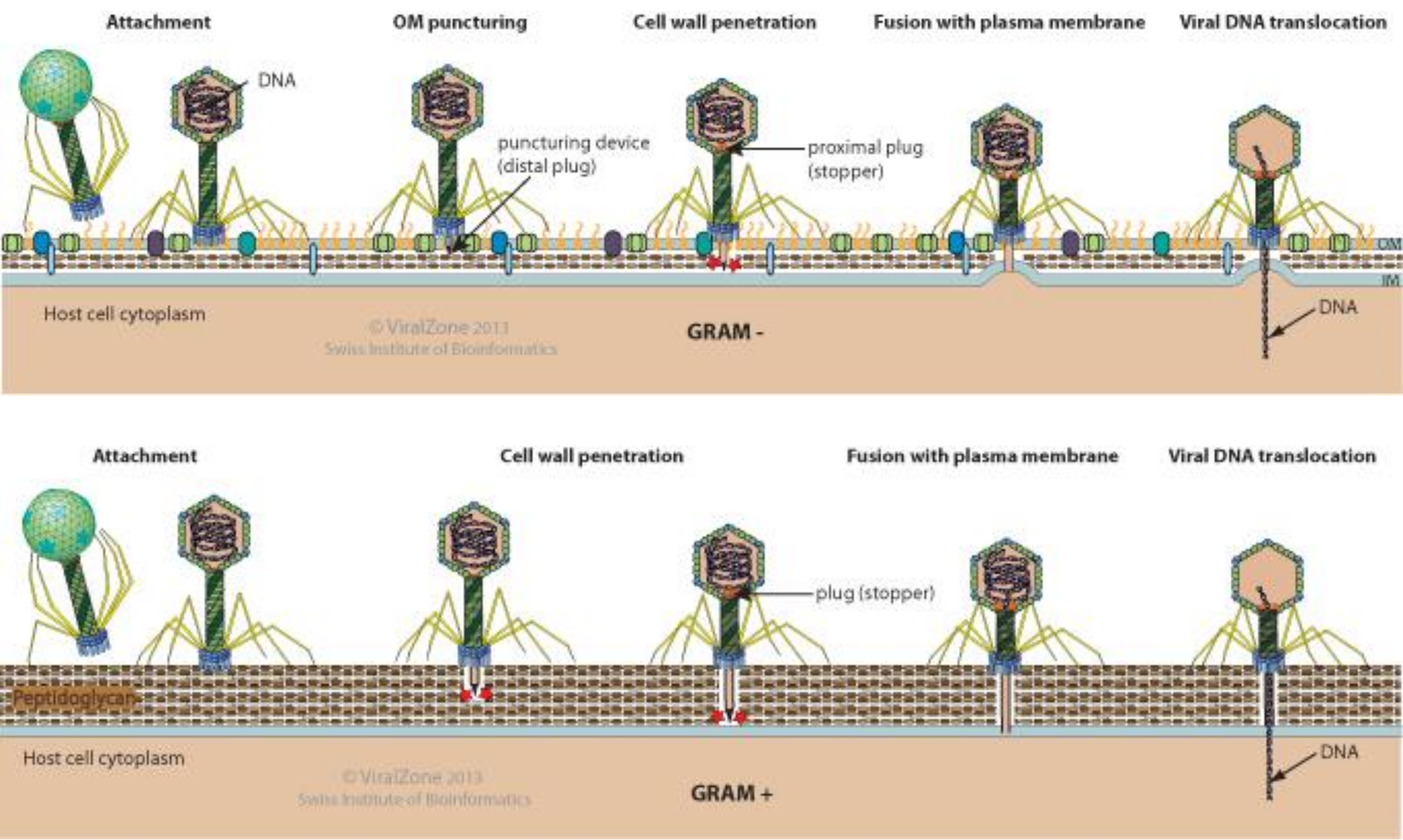


Genome ejection through host cell envelope

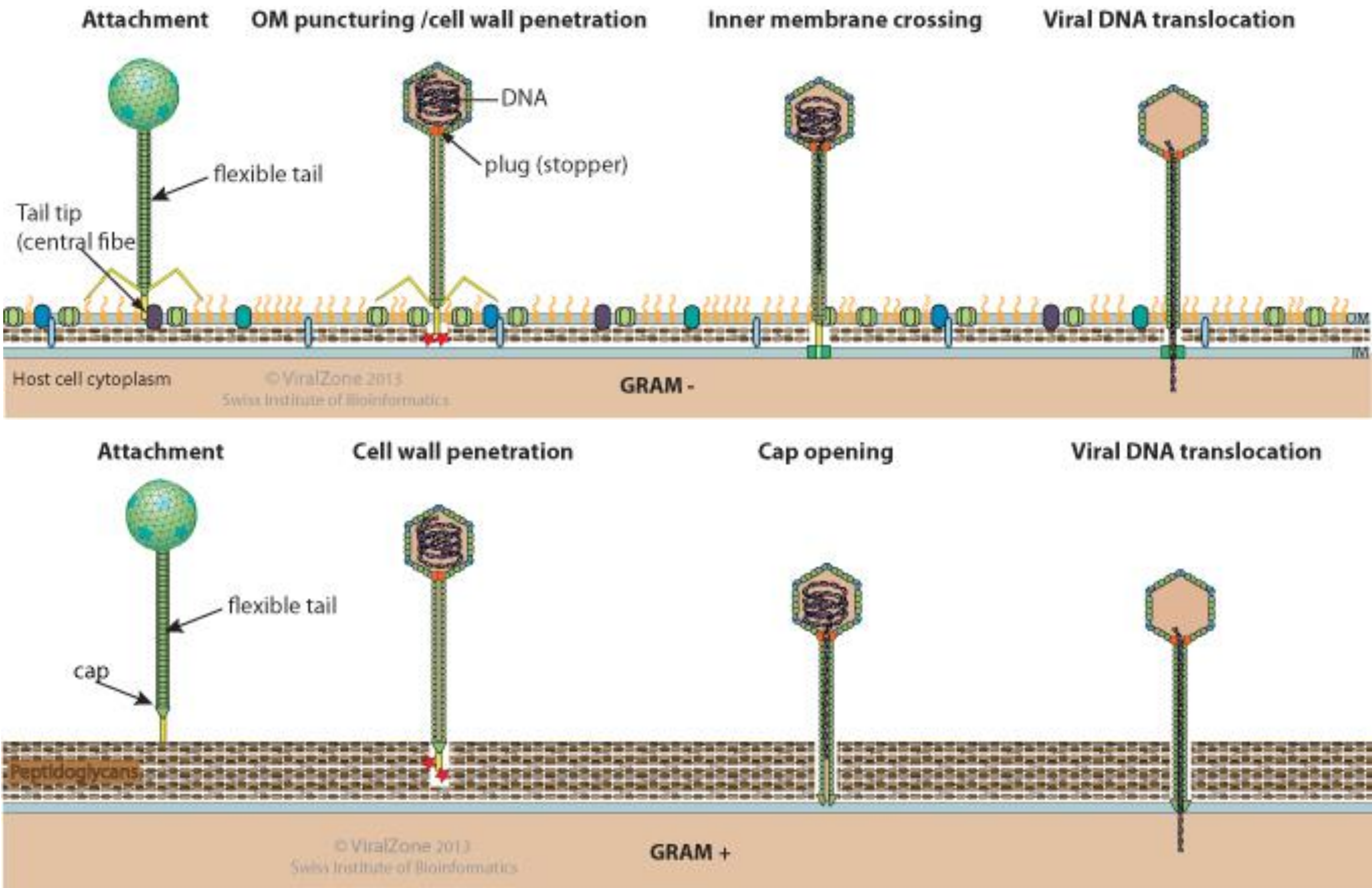


Myoviridae Siphoviridae Podoviridae

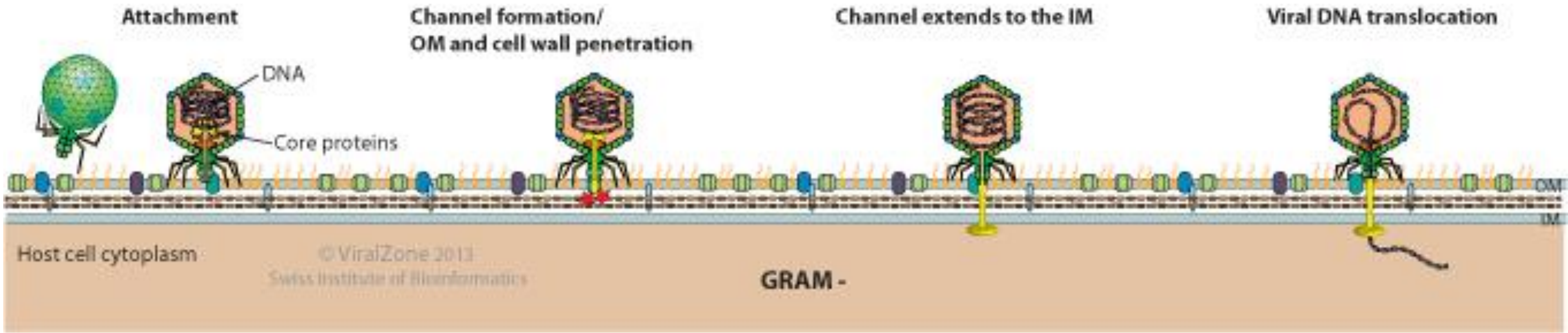
Viral contractile tail ejection system

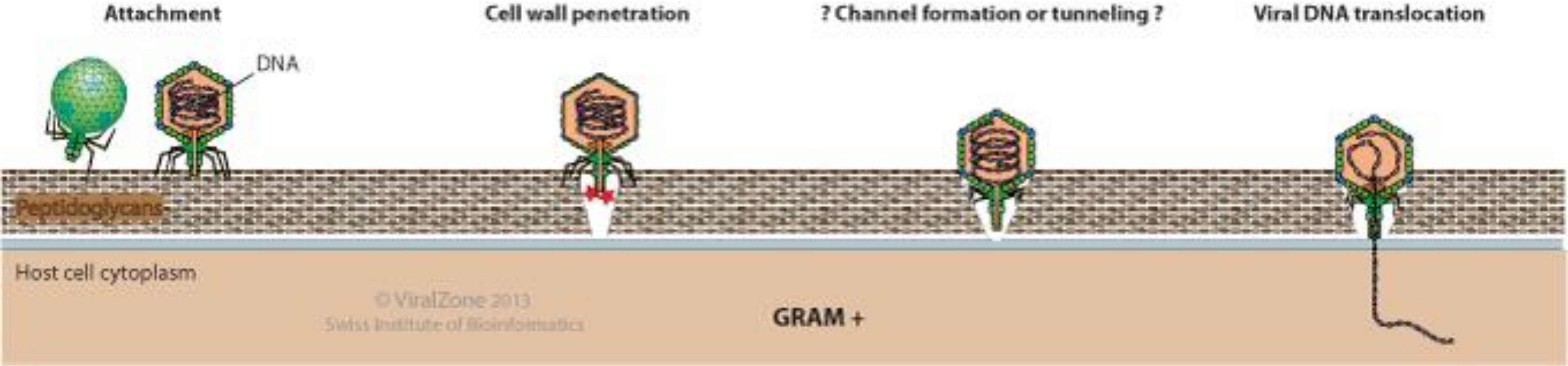


Viral long flexible tail ejection system



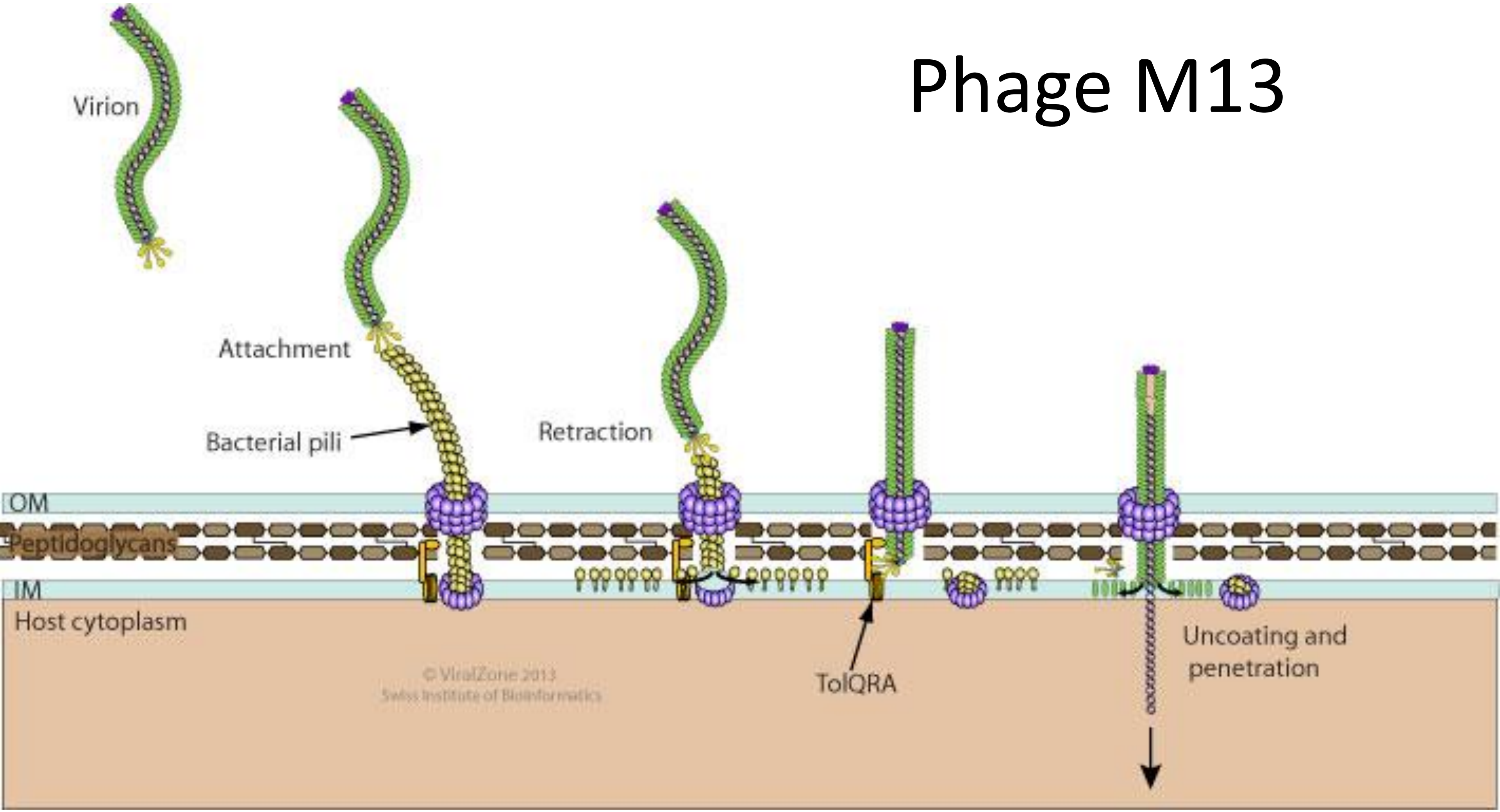
Viral short tail ejection system





Viral penetration into host cytoplasm via pilus retraction

Phage M13



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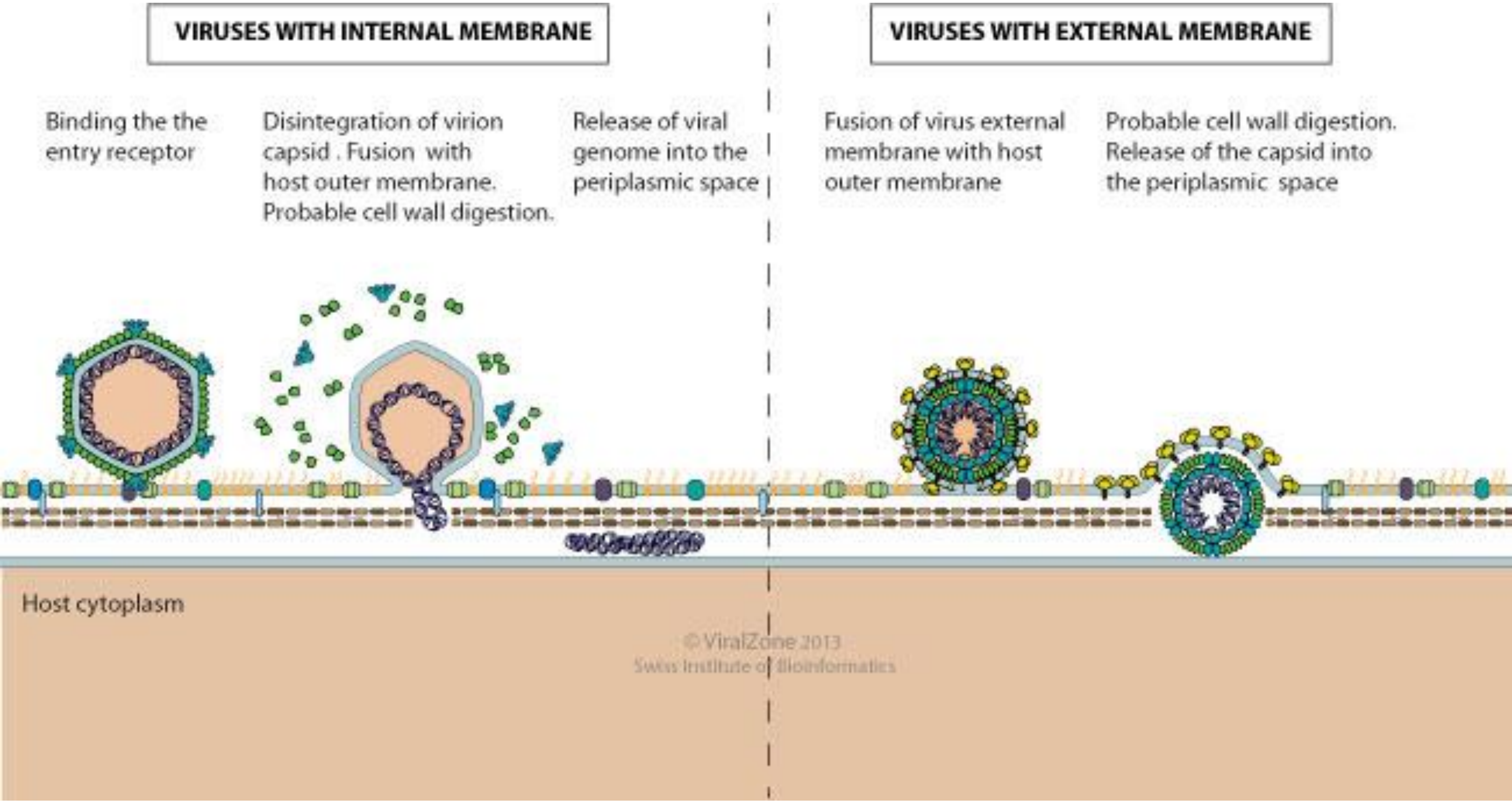
Fusion of virus membrane with host outer membrane



VIRUSES WITH INTERNAL MEMBRANE

Binding the the entry receptor

Disintegration of virion capsid . Fusion with host outer membrane. Probable cell wall digestion.

Release of viral genome into the periplasmic space

VIRUSES WITH EXTERNAL MEMBRANE

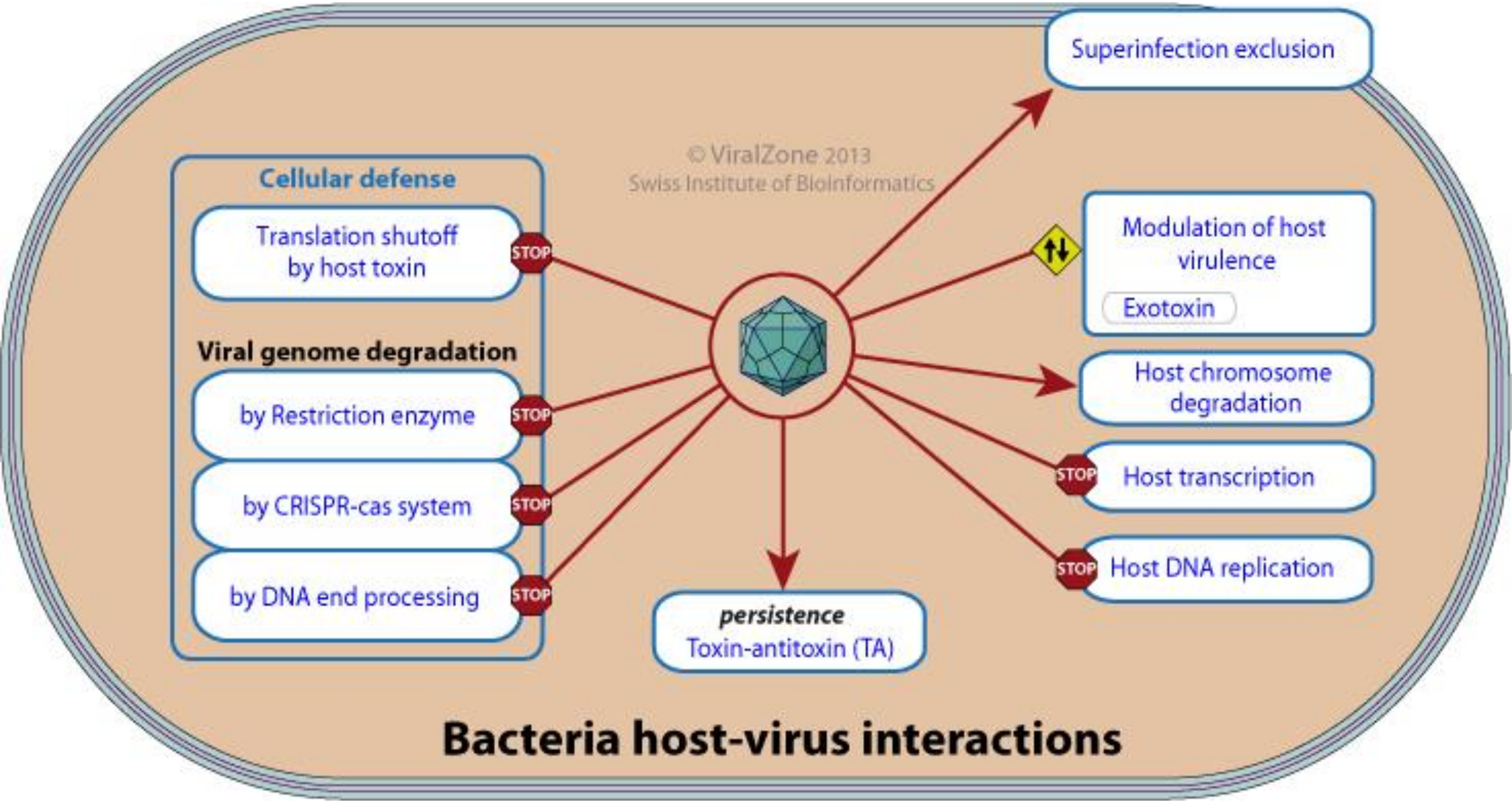
Fusion of virus external membrane with host outer membrane

Probable cell wall digestion. Release of the capsid into the periplasmic space

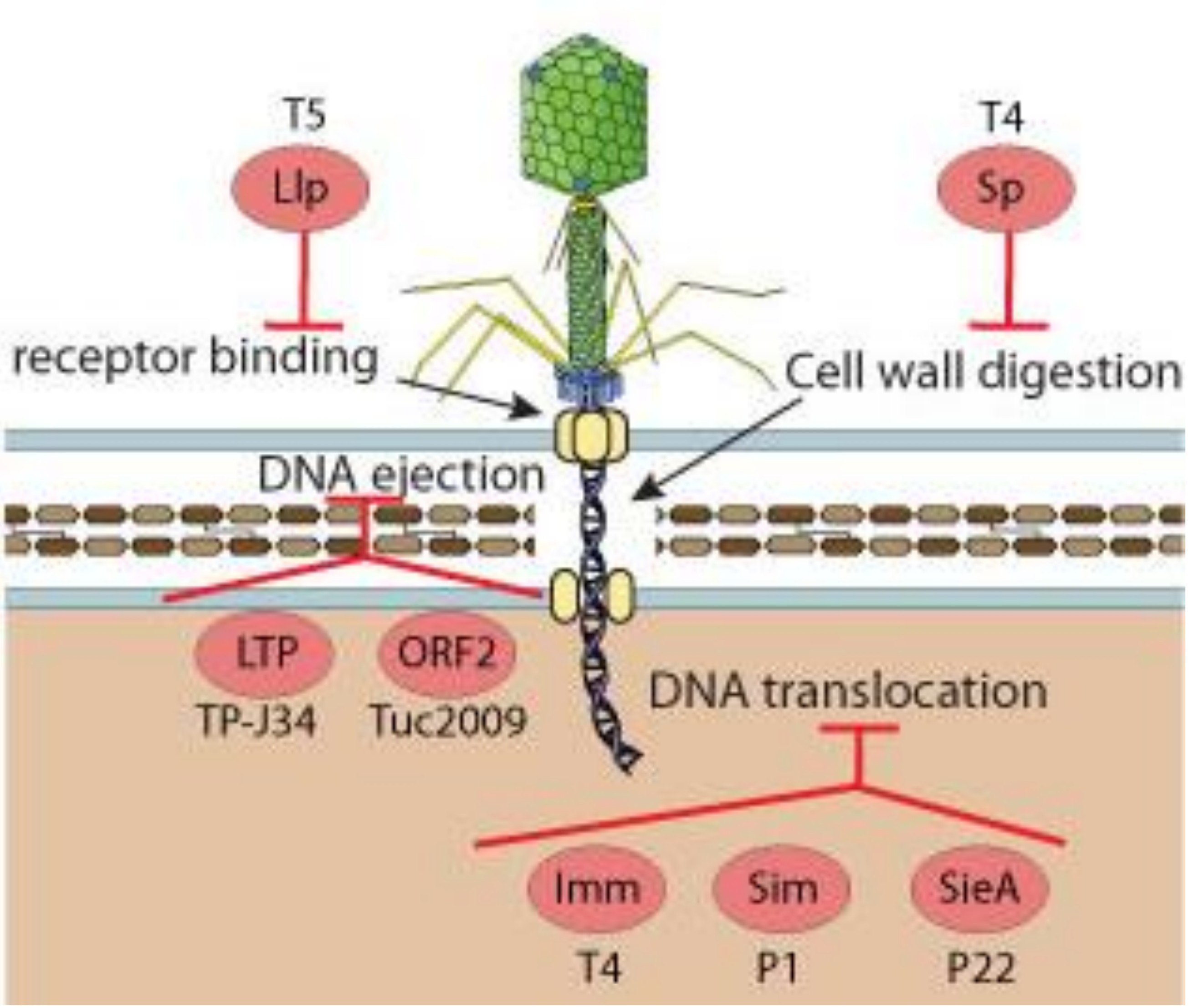
Cystoviridae

Corticoviridae

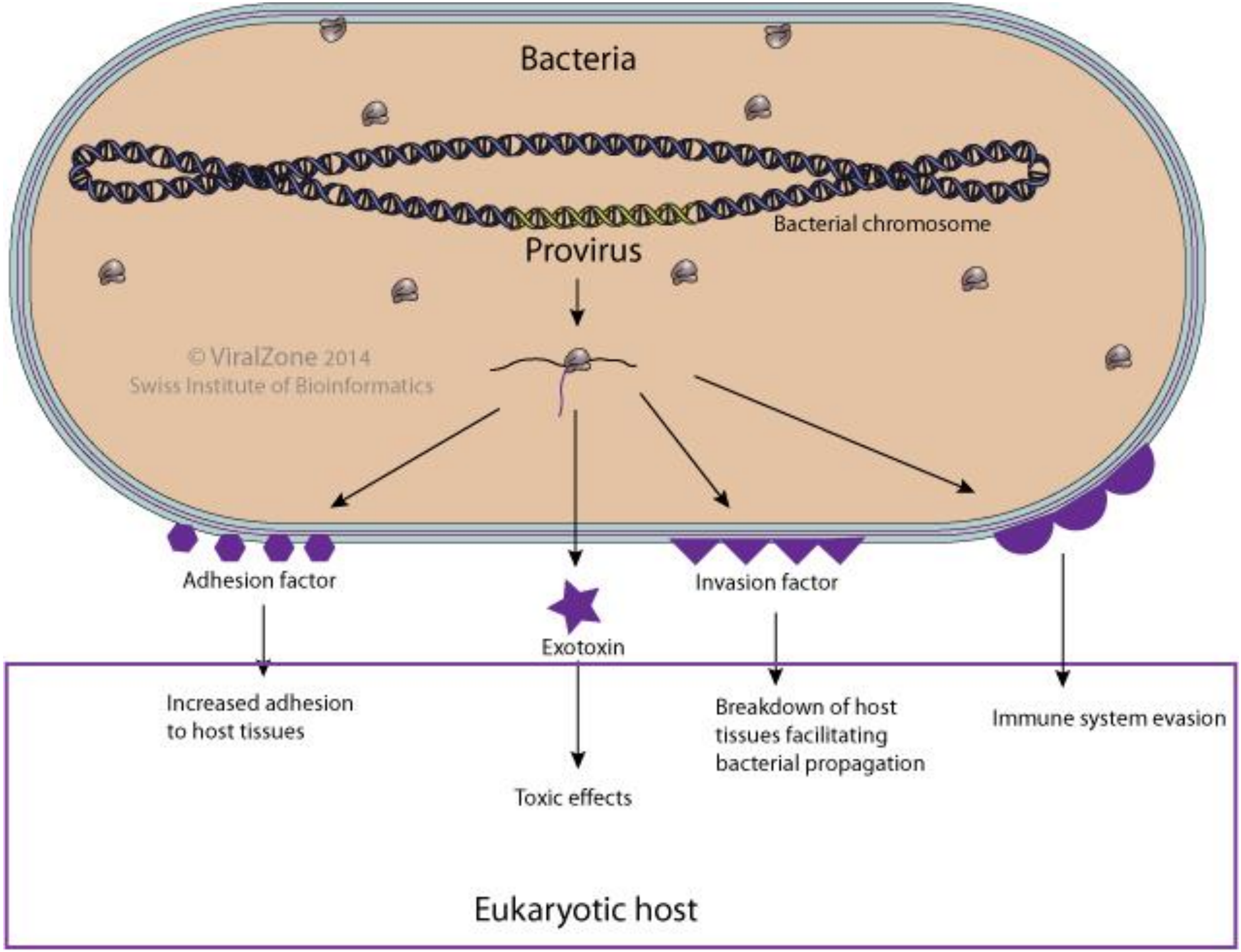
Phage-bacteria interactions



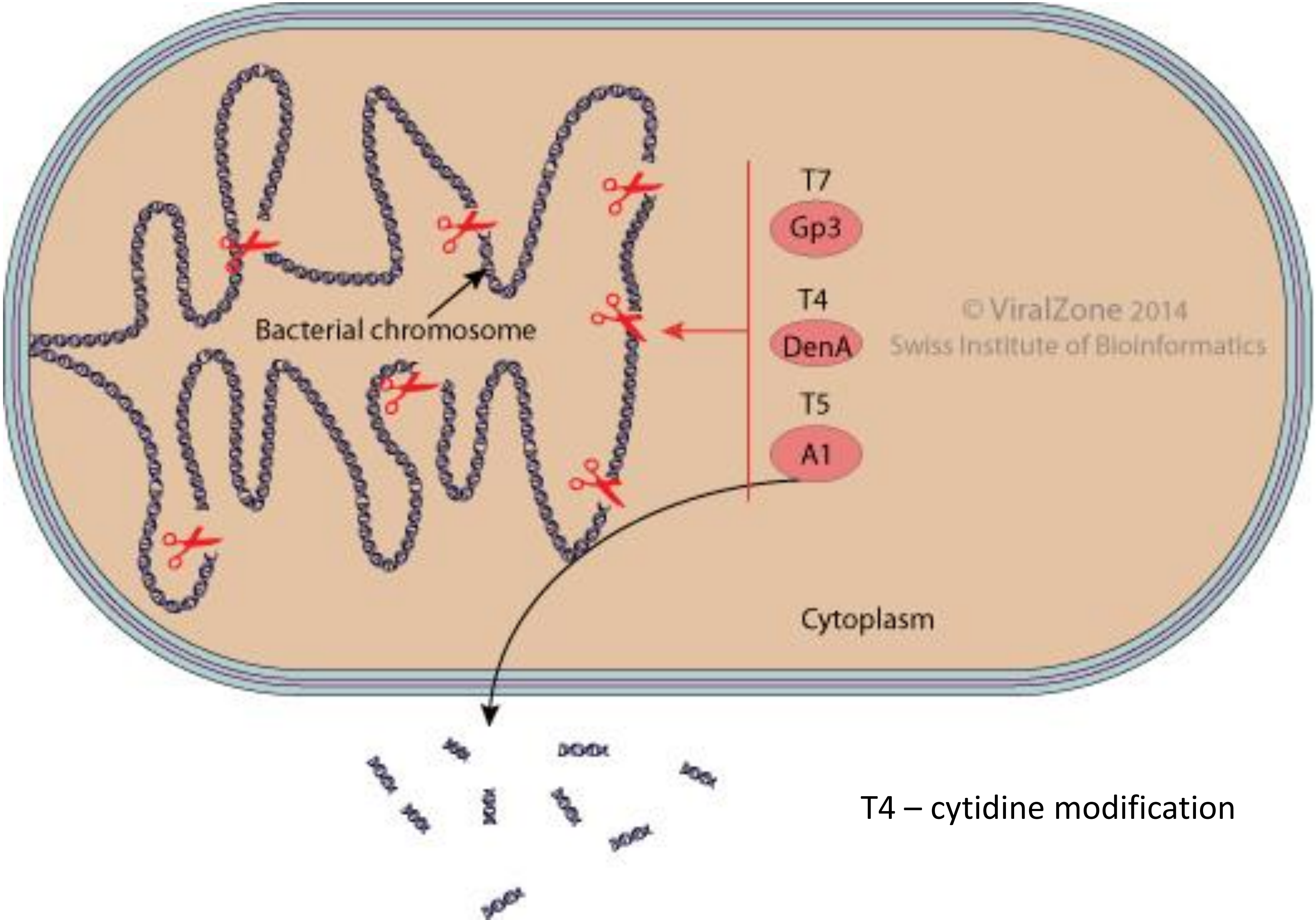
Superinfection exclusion



Modulation of host host virulence by virus

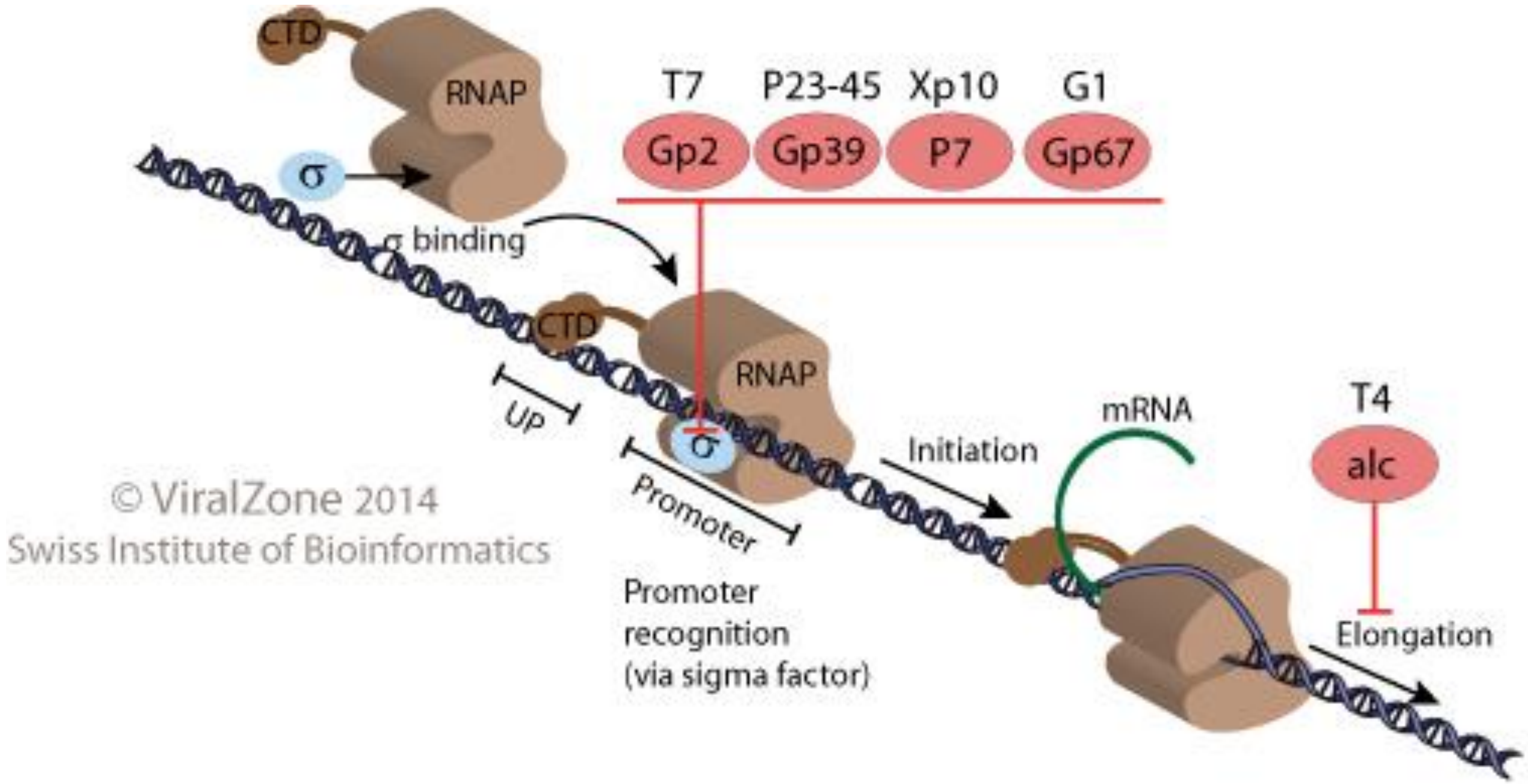


Degradation of host chromosome by phage



T4 – cytidine modification

Phage host transcription shutoff



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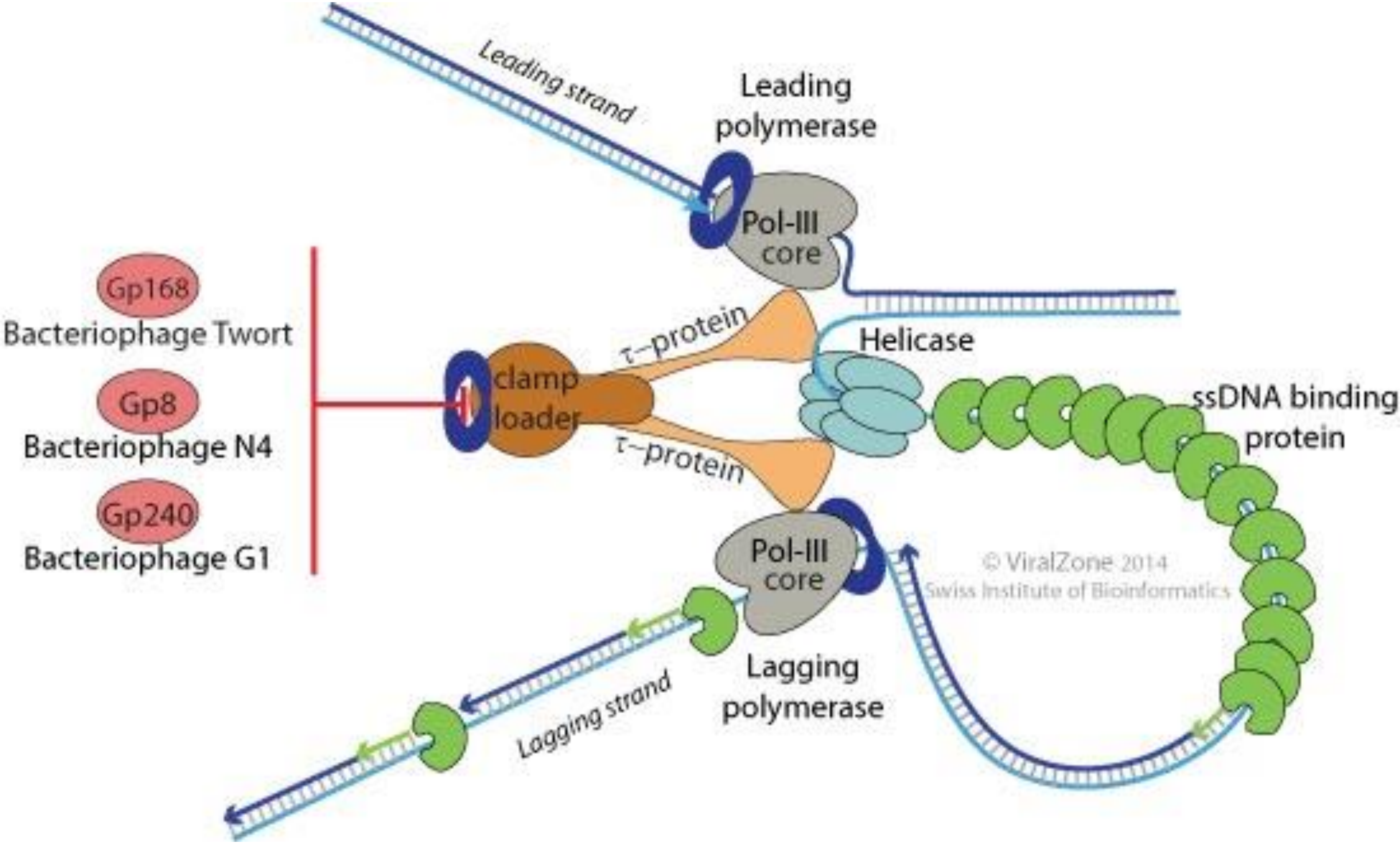
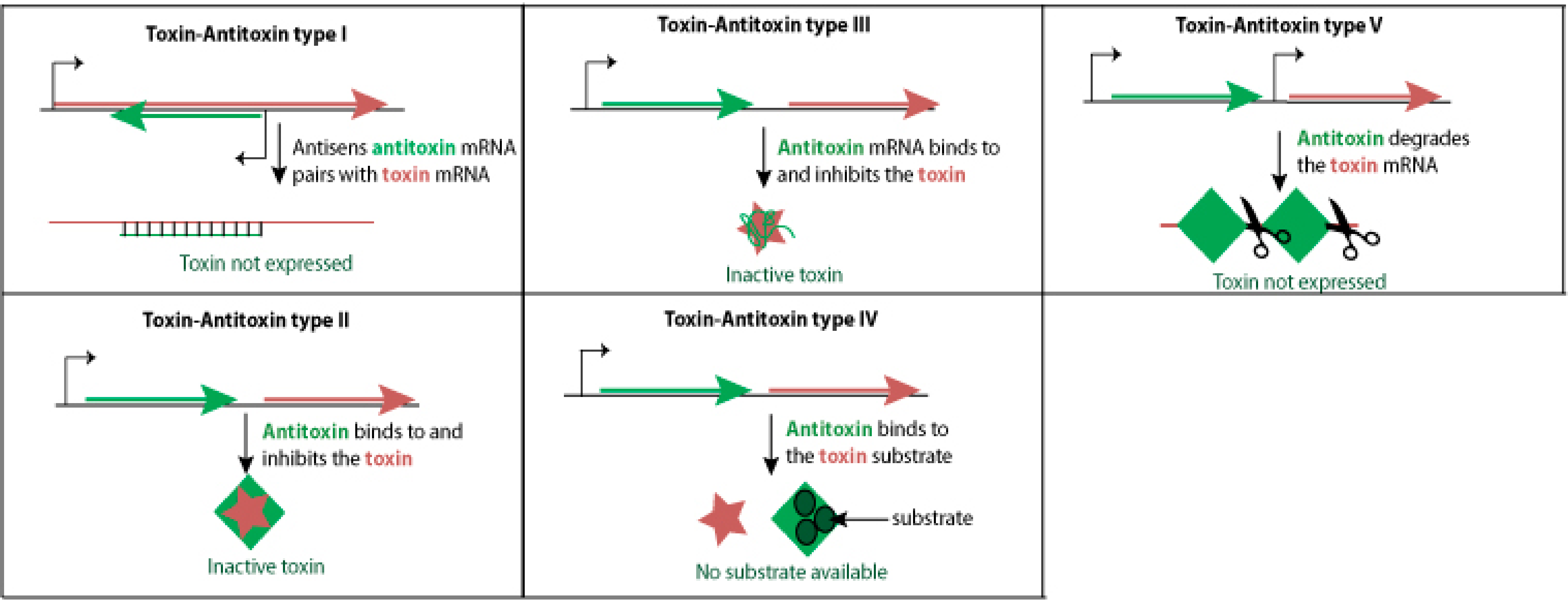
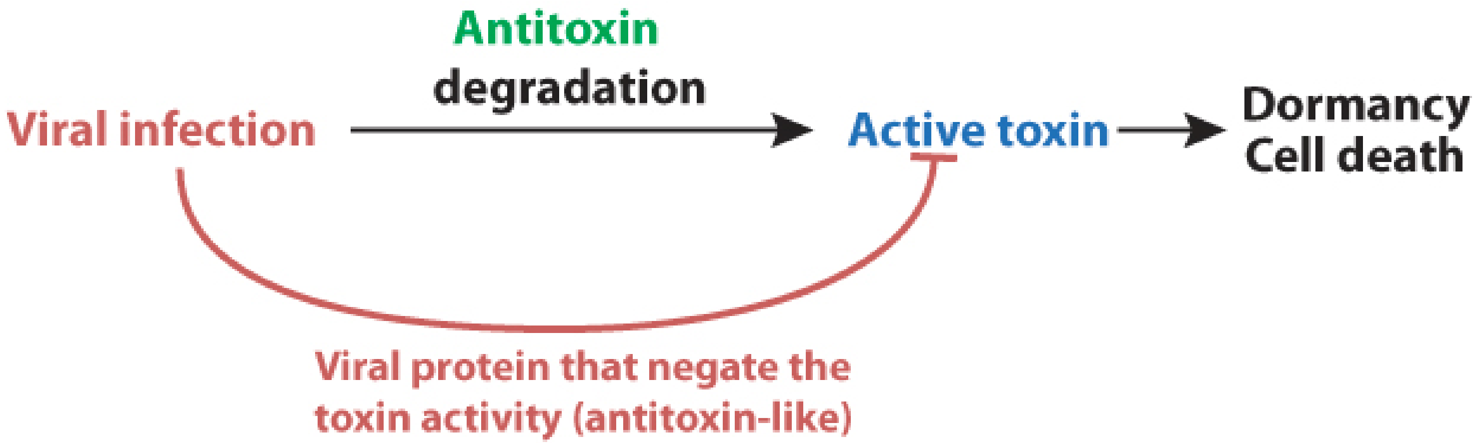
Inhibition of host DNA replication by virus



Toxin-antitoxin systems as antiviral defense



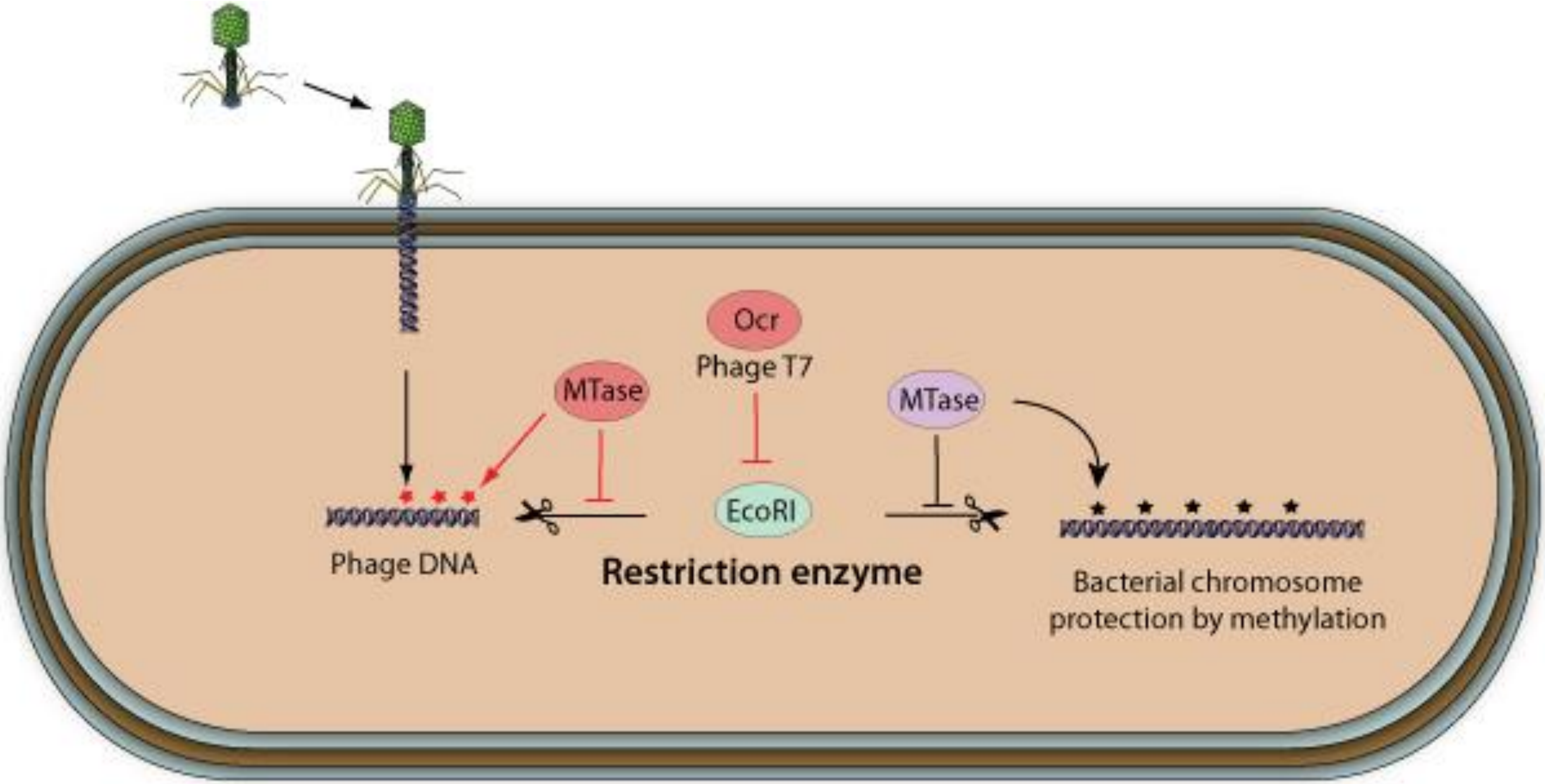


Antitoxin degradation

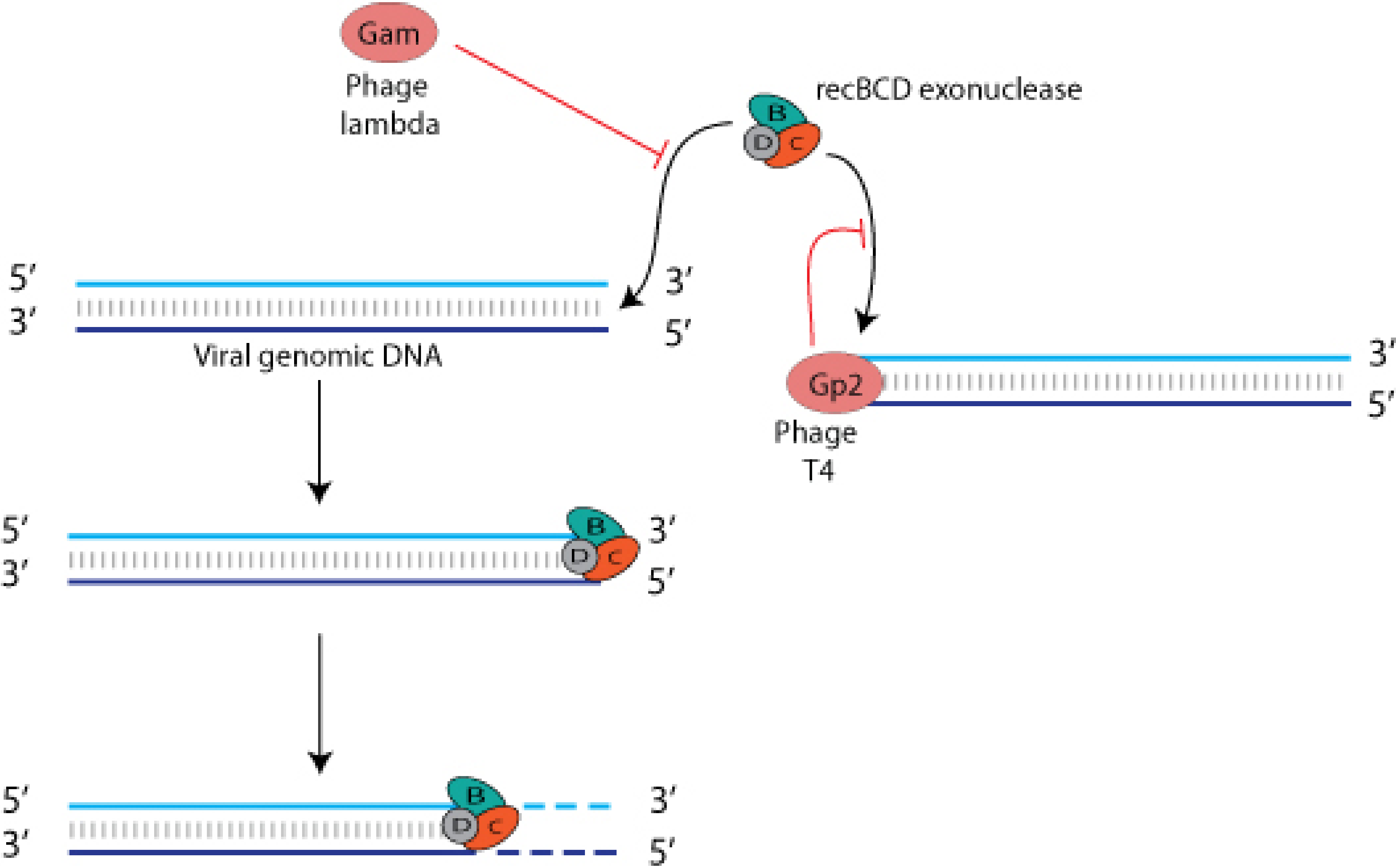
Viral infection → **Active toxin** → **Dormancy Cell death**

Viral protein that negate the toxin activity (antitoxin-like)

Restriction-modification system evasion by virus

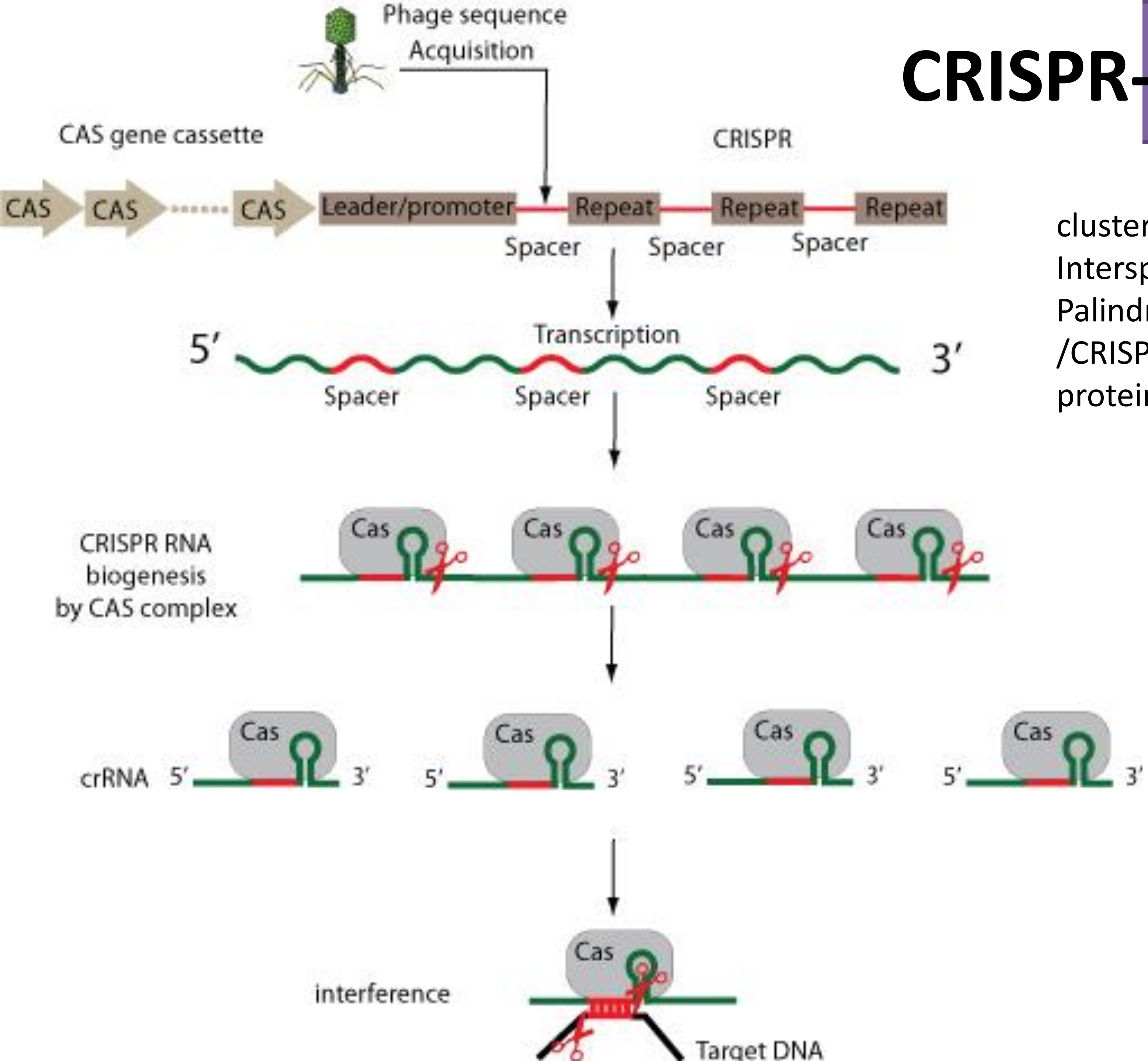


DNA end degradation evasion by virus

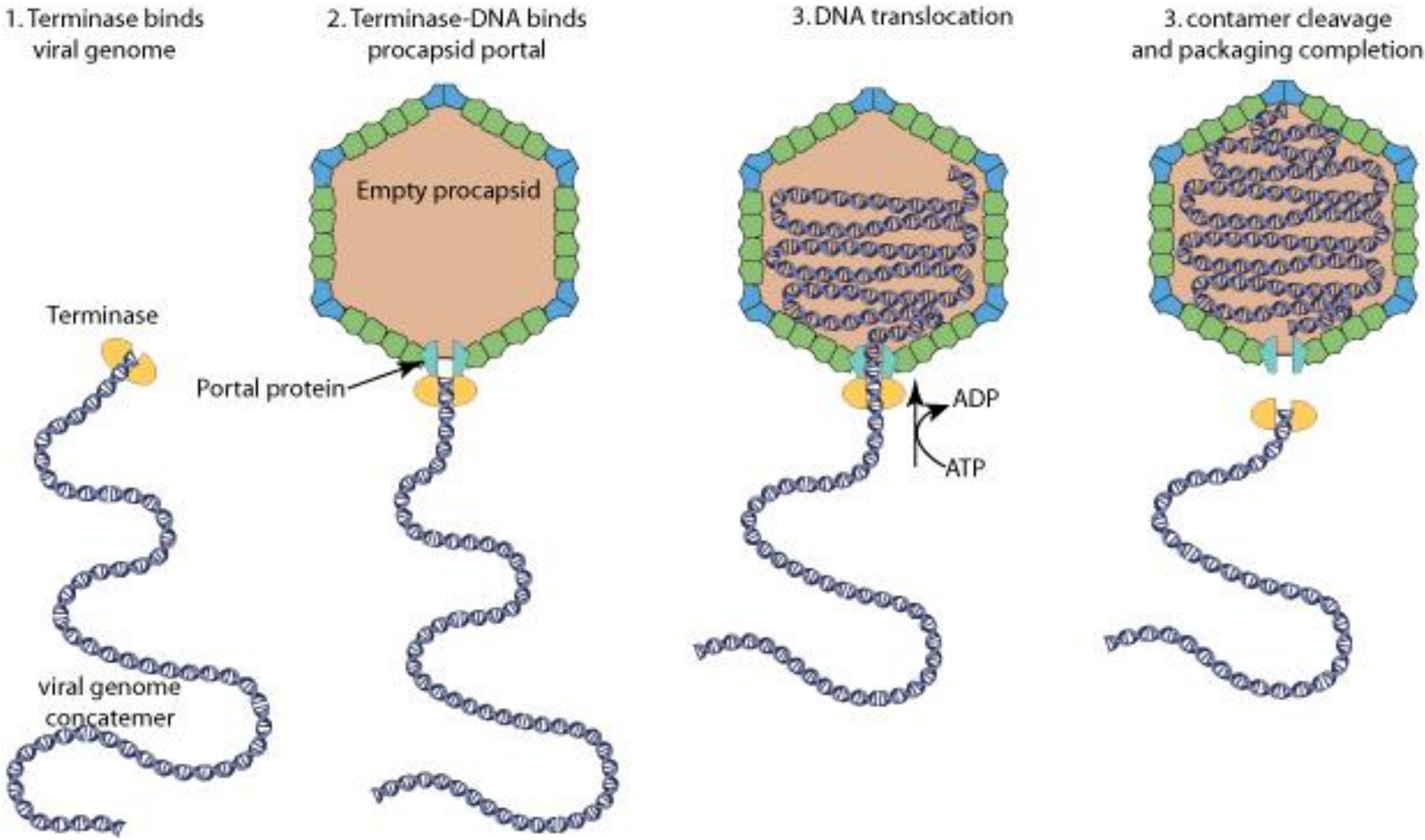


CRISPR-cas

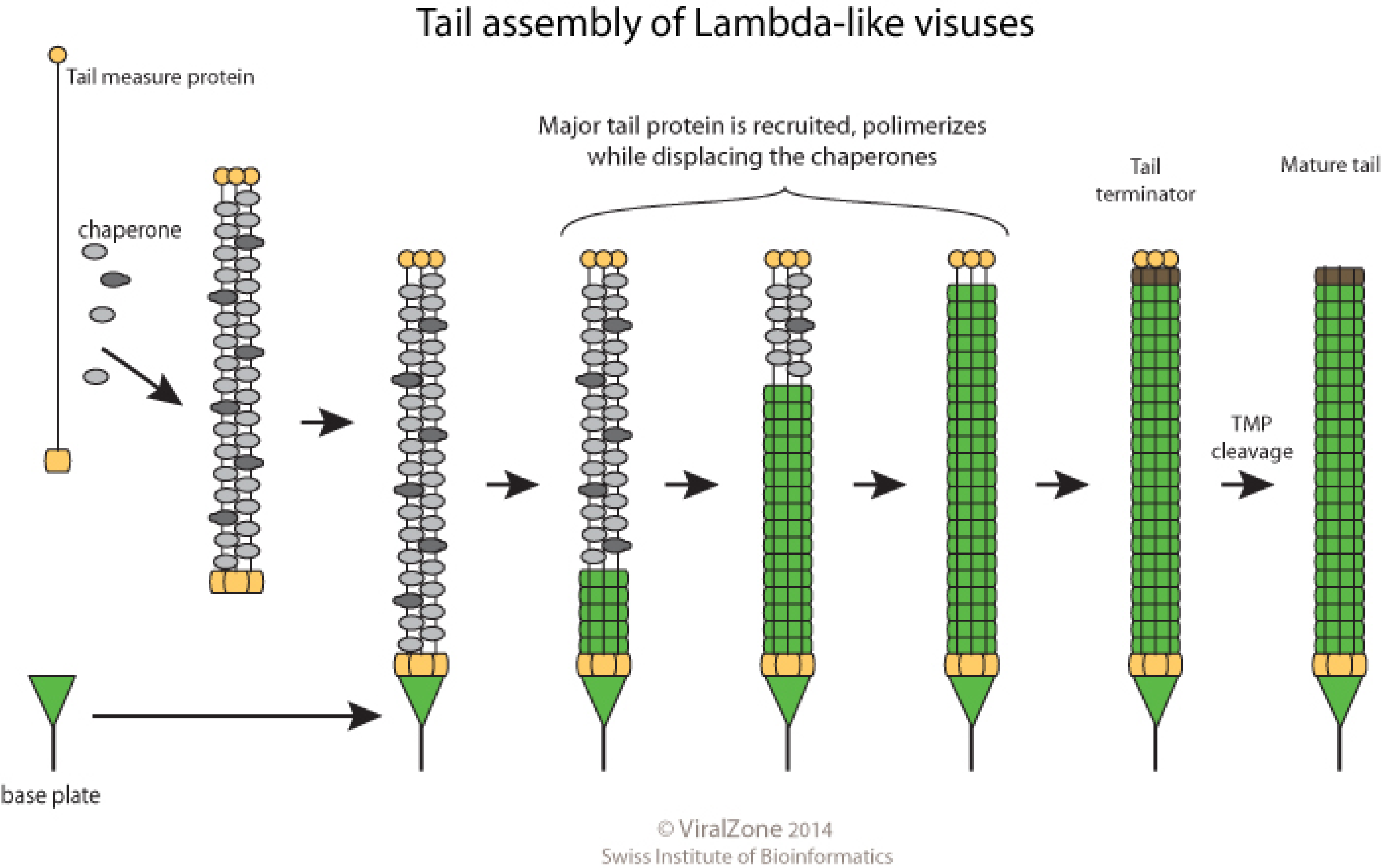
clustered Regularly Interspaced Short Palindromic Repeats /CRISP-associated proteins



Phage genome packaging



Tail assembly



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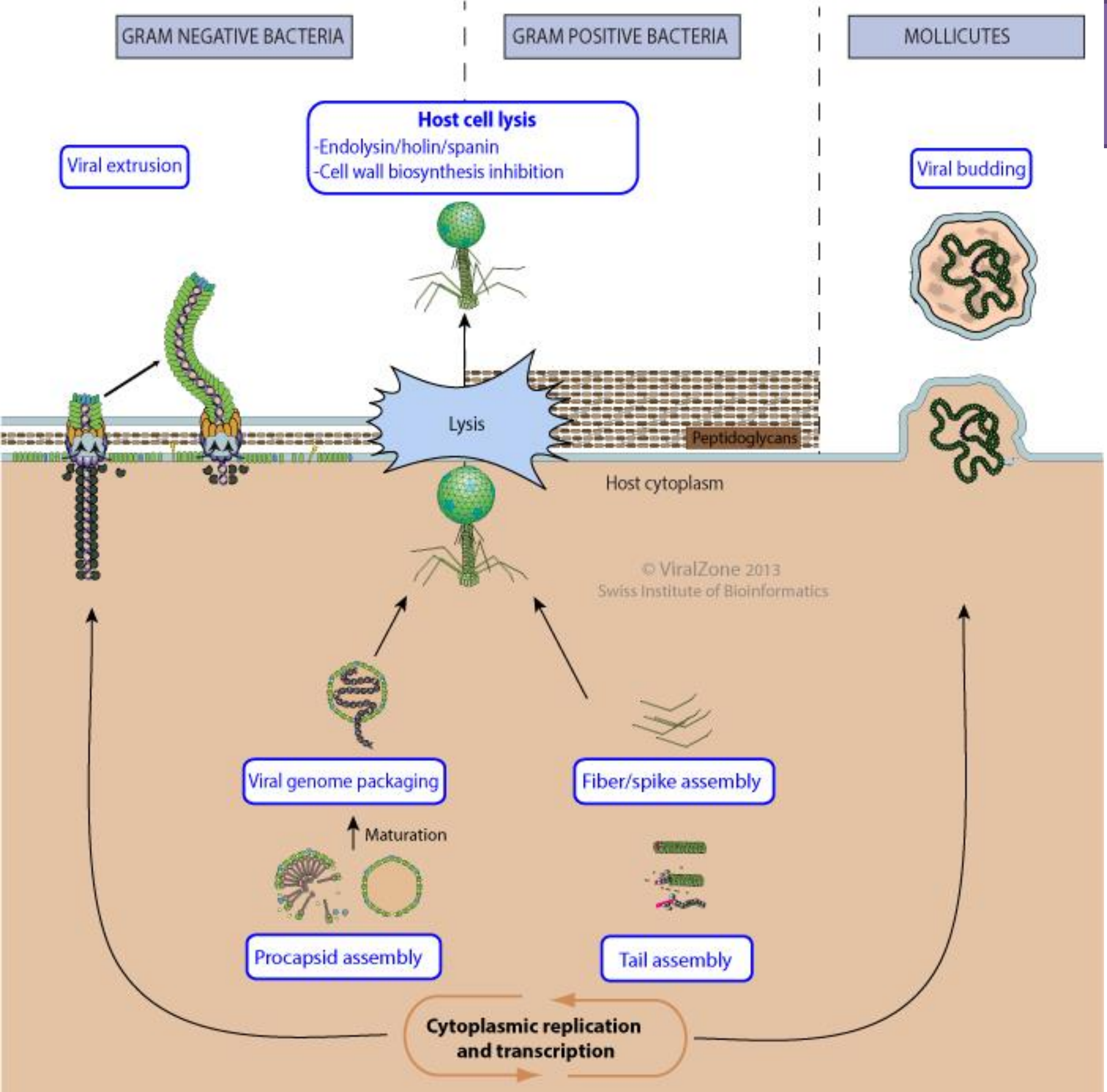
Phage exit


GRAM NEGATIVE BACTERIA
GRAM POSITIVE BACTERIA
MOLLICUTES
Viral extrusion
Host cell lysis
-Endolysin/holin/spanin
-Cell wall biosynthesis inhibition
Viral budding
Lysis
Peptidoglycans
Host cytoplasm
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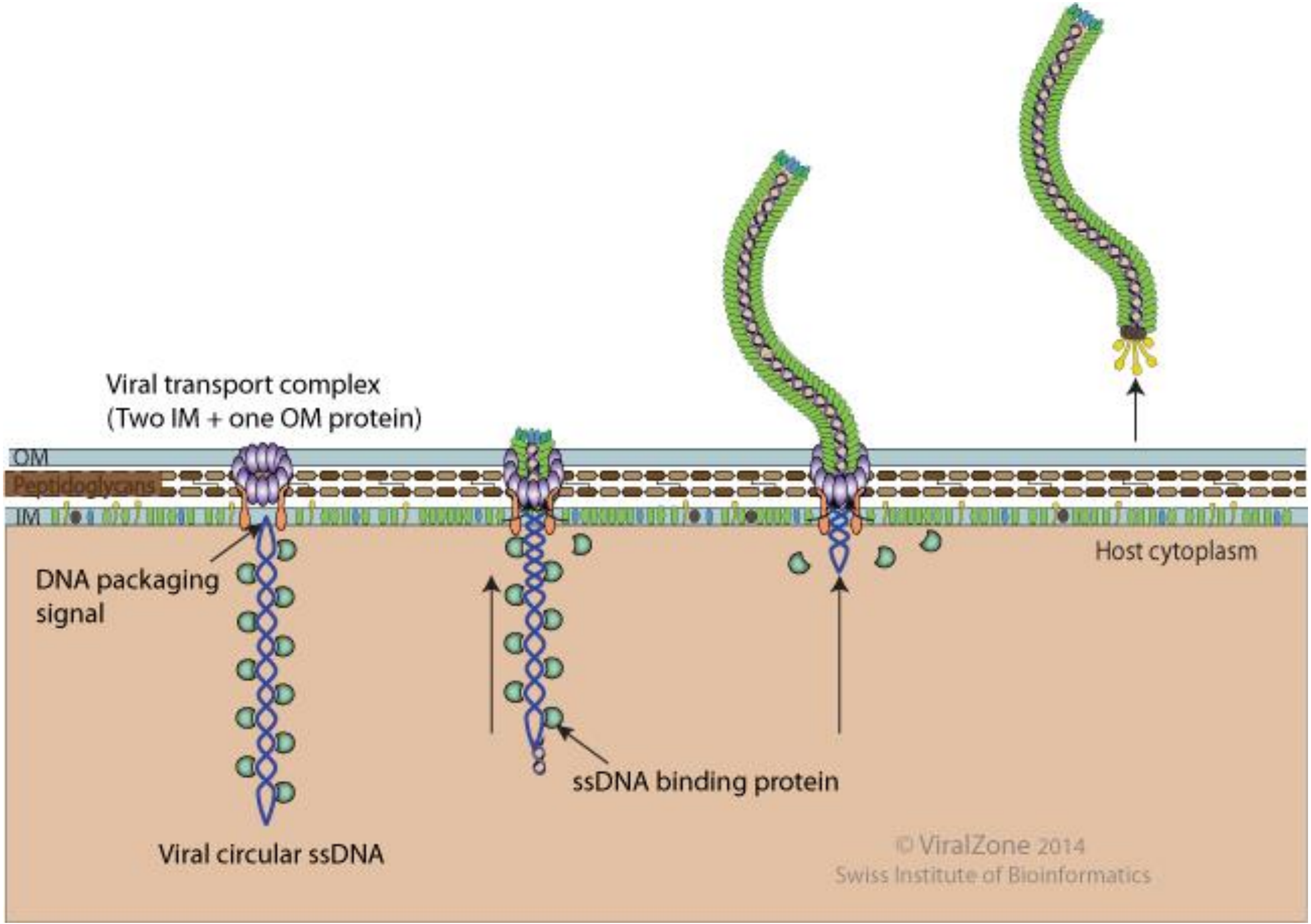
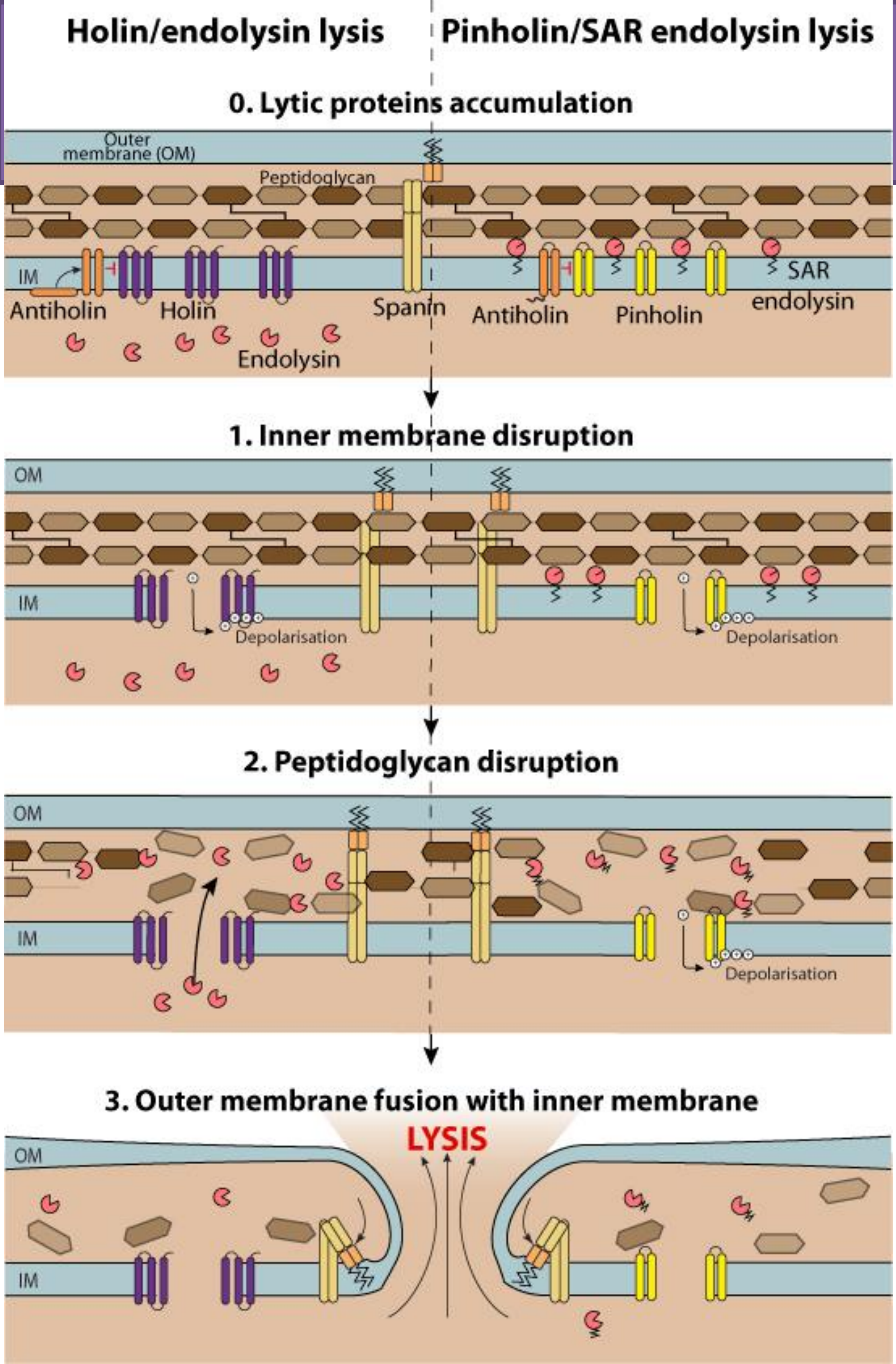
Viral genome packaging
Fiber/spike assembly
Maturation
Procapsid assembly
Tail assembly
Cytoplasmic replication and transcription

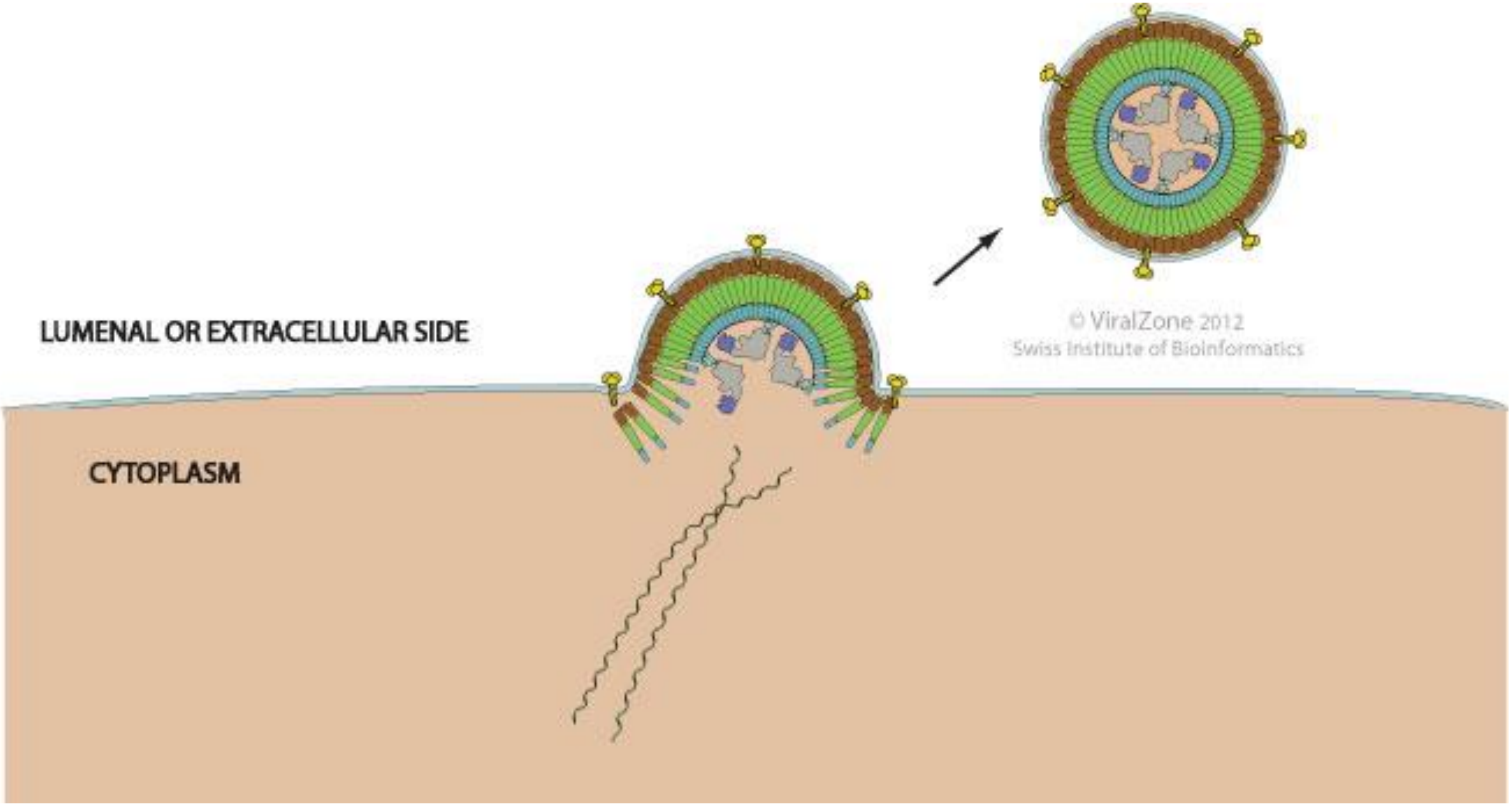
Phage extrusion





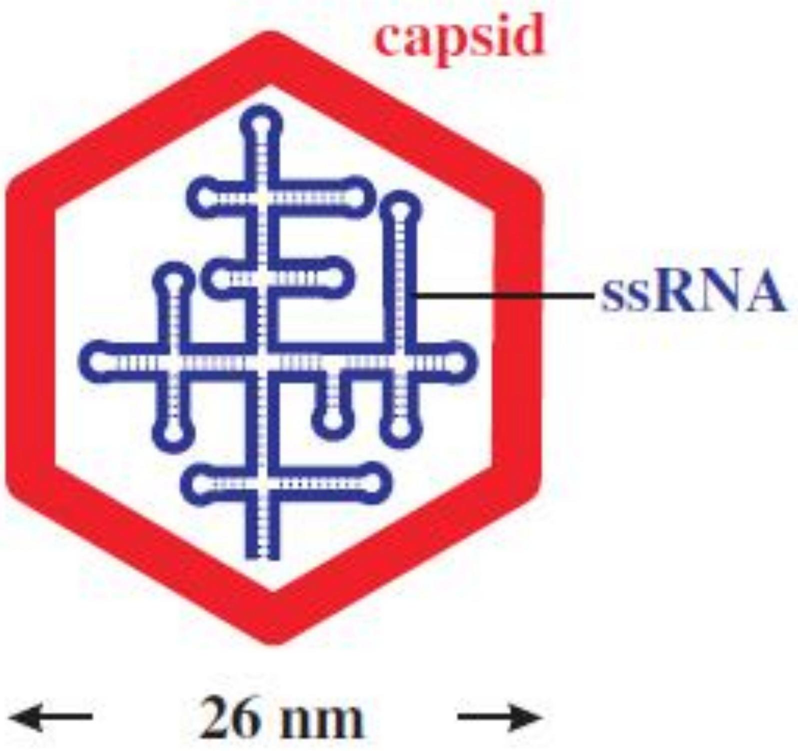
Holin/endolysin/spanin cell lysis by phage

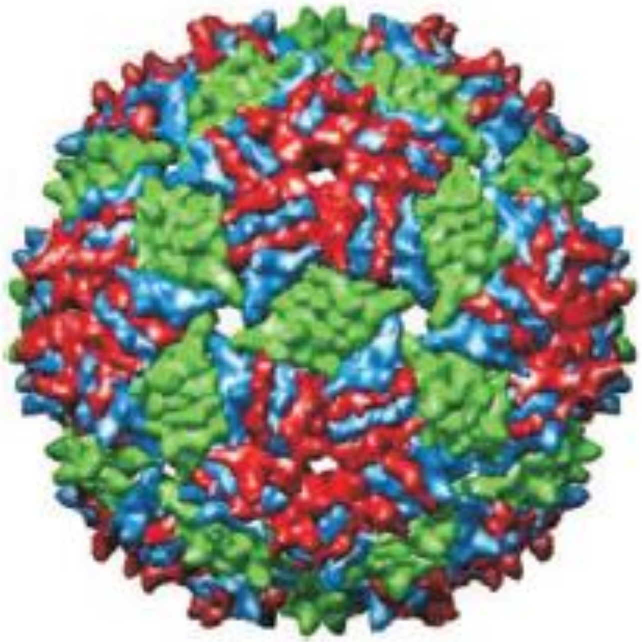
Phage budding



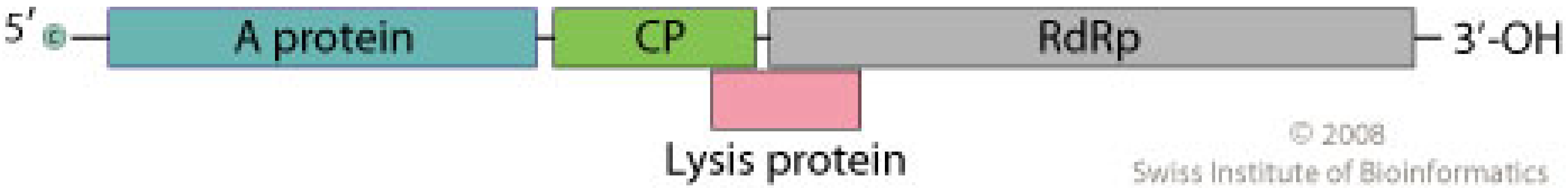
Plasmaviridae

Bacteriophage MS2





Enterobacteria phage MS2



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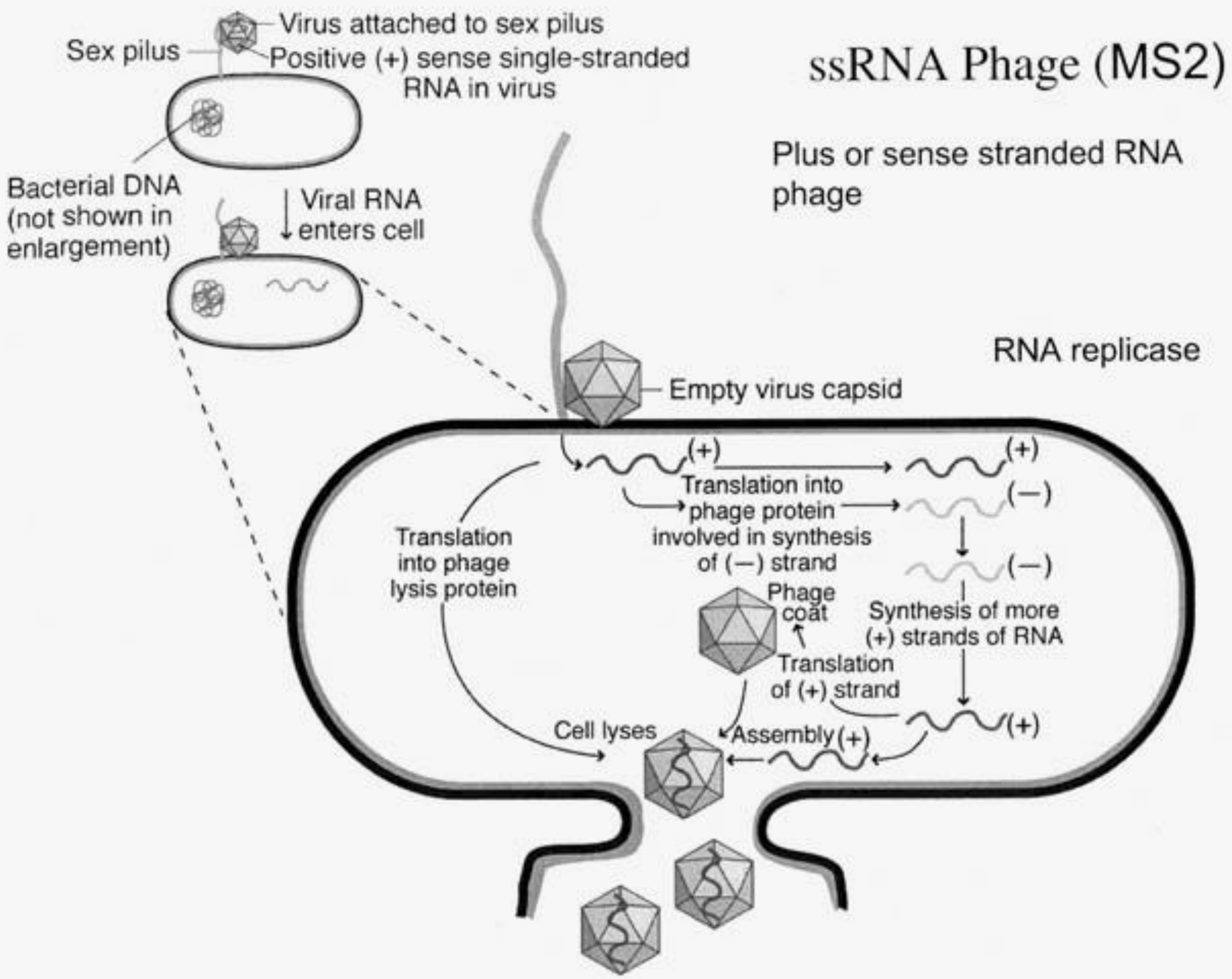
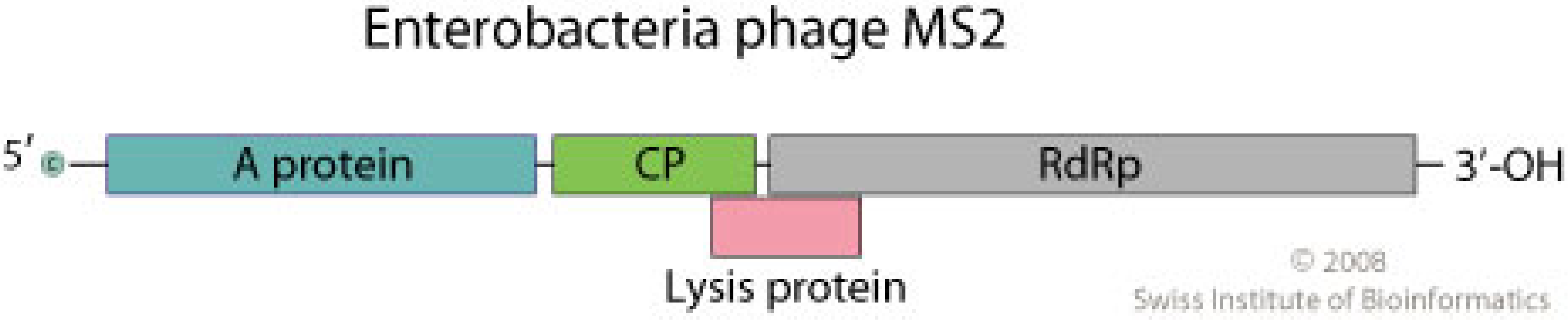
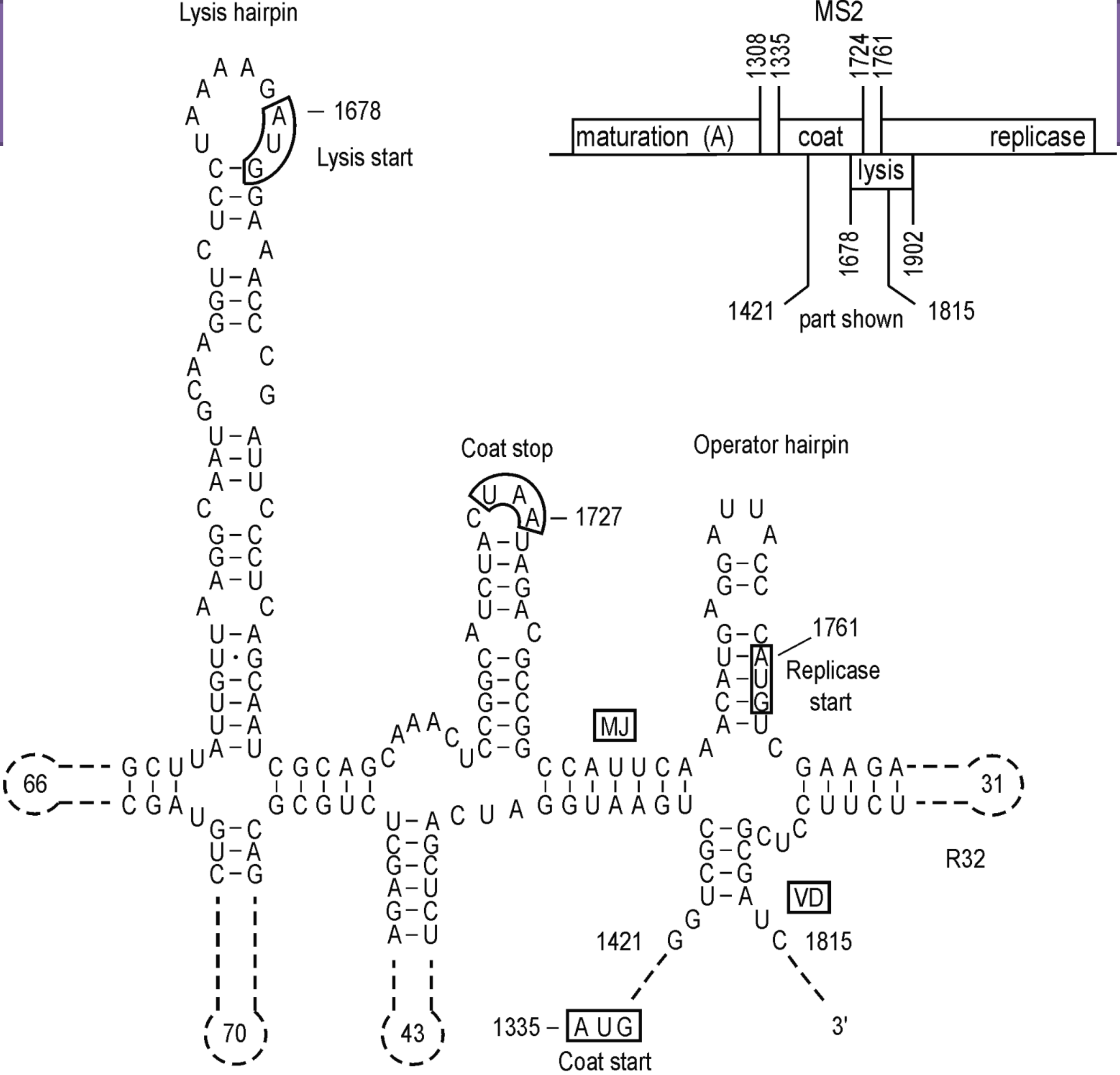
MS2 life-cycle



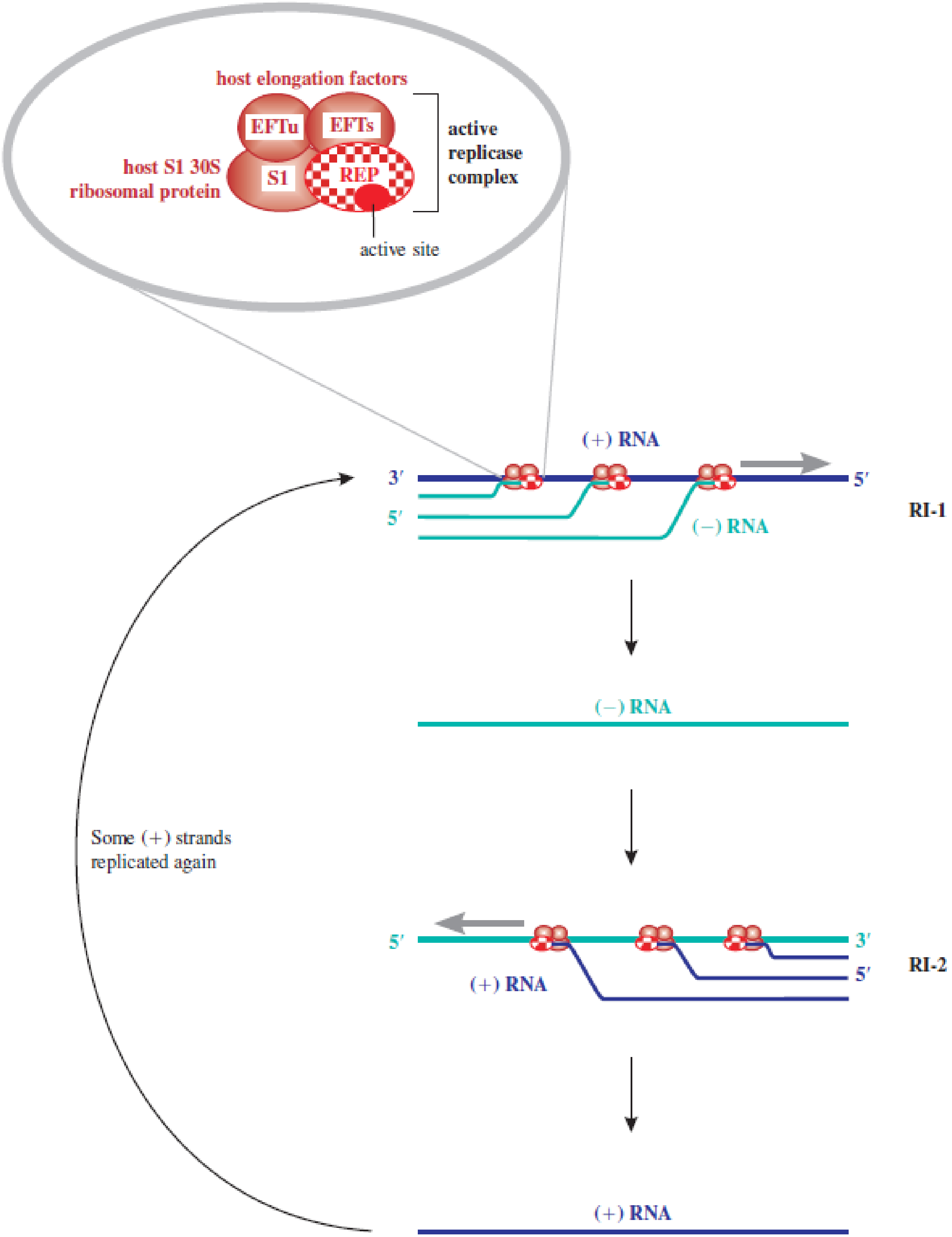
Leviviridae gene expression regulation

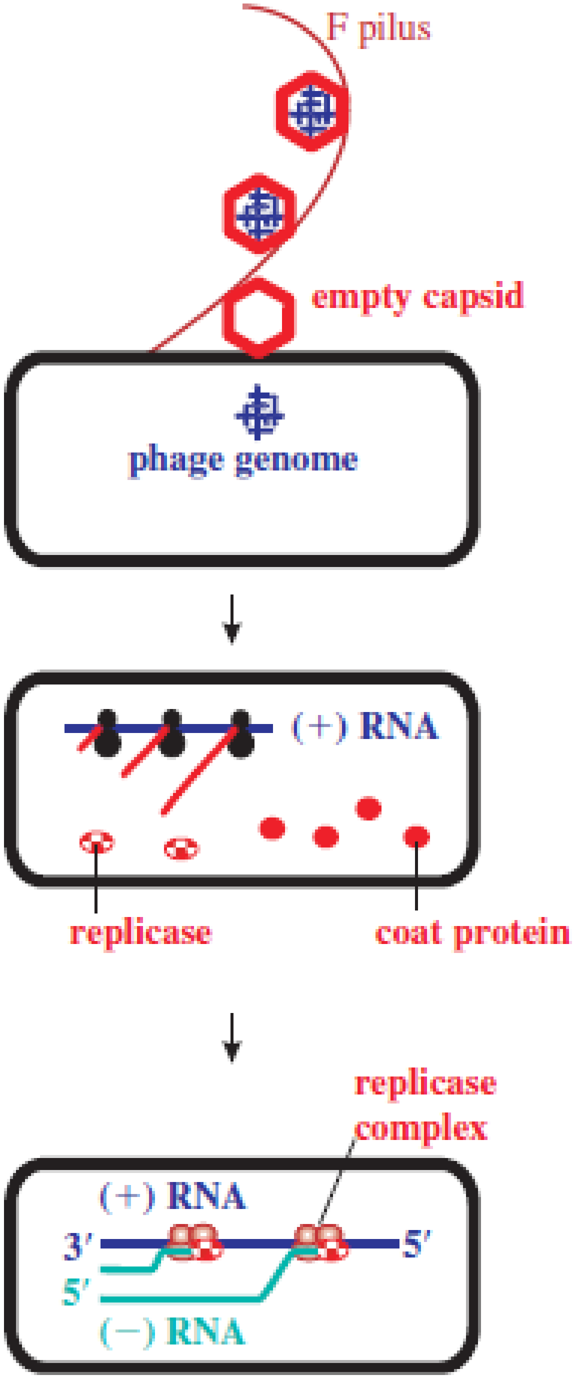


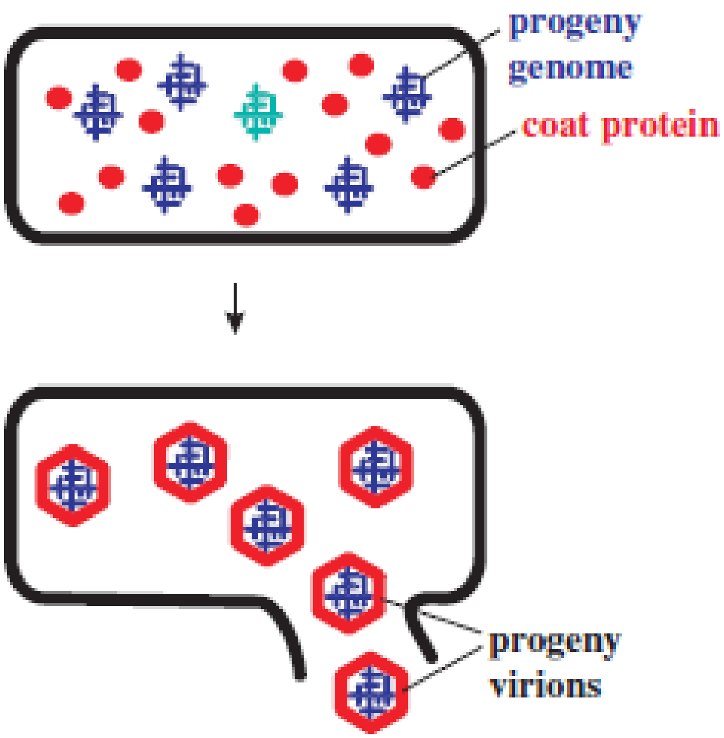
The gene for the most abundant protein, the coat protein, can be immediately translated.
The translation start of the replicase gene is normally hidden within RNA secondary structure, but can be transiently opened as ribosomes pass through the coat protein gene.
Replicase translation is also shut down once large amounts of coat protein have been made; coat protein dimers bind and stabilize the RNA "operator hairpin", blocking the replicase start.
The start of the maturation protein gene is accessible in RNA being replicated but hidden within RNA secondary structure in the completed MS2 RNA; this ensures translation of only a very few copies of maturation protein per RNA.
The lysis protein gene can only be initiated by ribosomes that have completed translation of the coat protein gene and "slip back" to the start of the lysis protein gene, at about a 5% frequency.


Lysis hairpin
1678
Lysis start
MS2
1308
1335
1724
1761
maturation (A)
coat
replicase
lysis
1678
1902
1421
part shown
1815
Coat stop
1727
Operator hairpin
1761
Replicase start
MJ
VD
R32
66
70
43
31
1421
1815
1335 – AUG
Coat start
3'

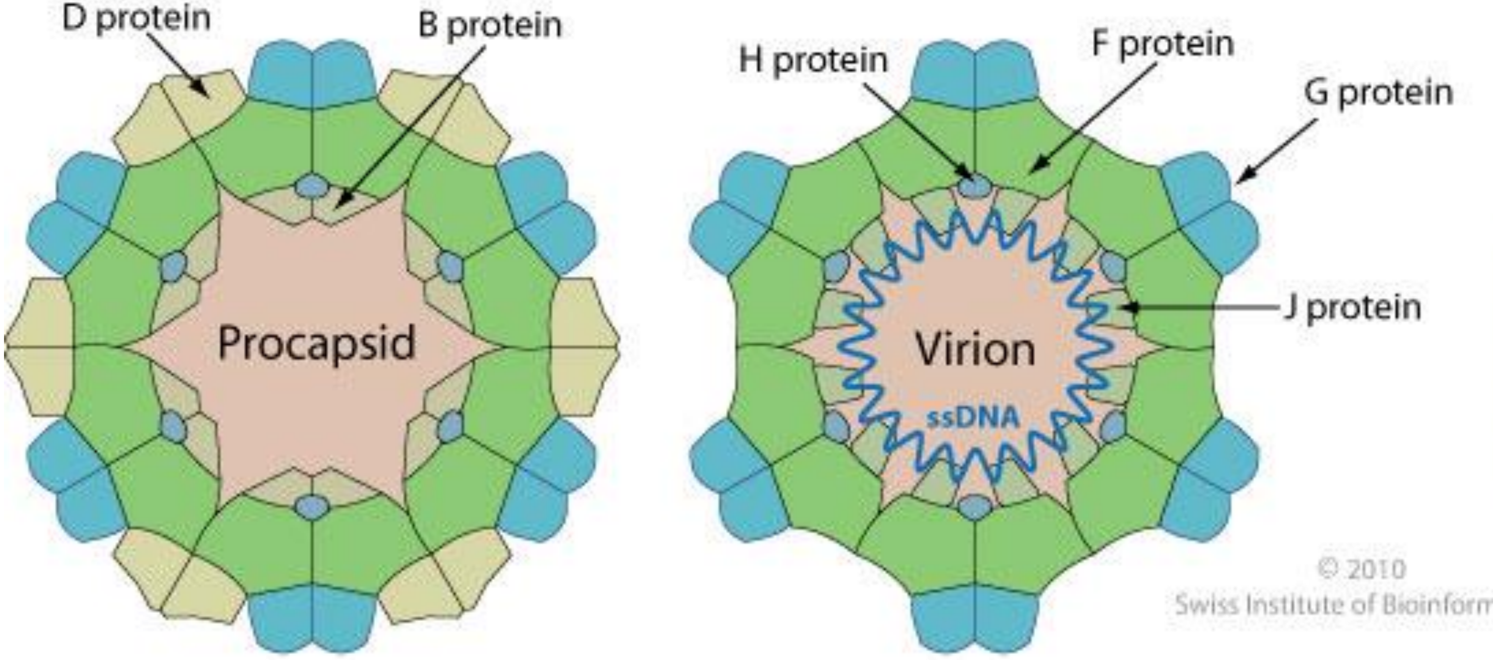
Leviviridae replication




F pilus
empty capsid
phage genome
(+) RNA
replicase
coat protein
replicase complex
(+) RNA
3′
5′
5′
(−) RNA


progeny genome
coat protein
progeny virions

phiX174



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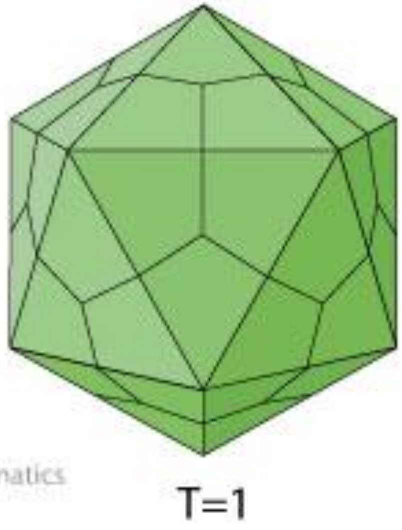
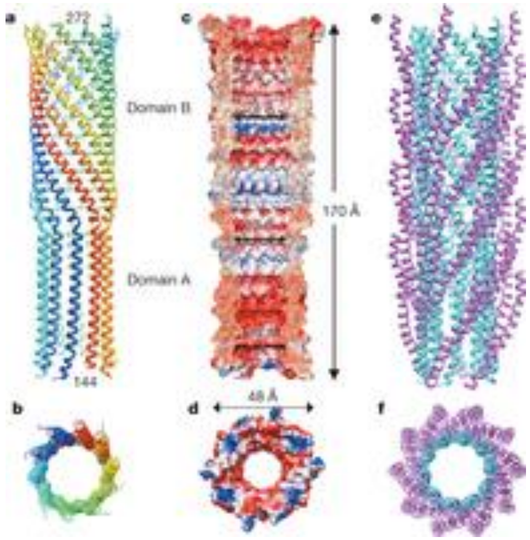
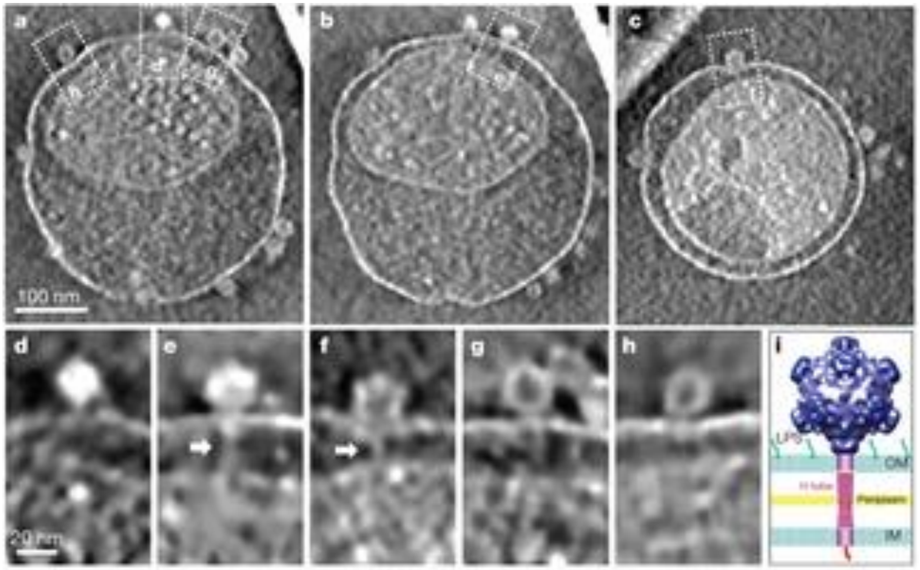
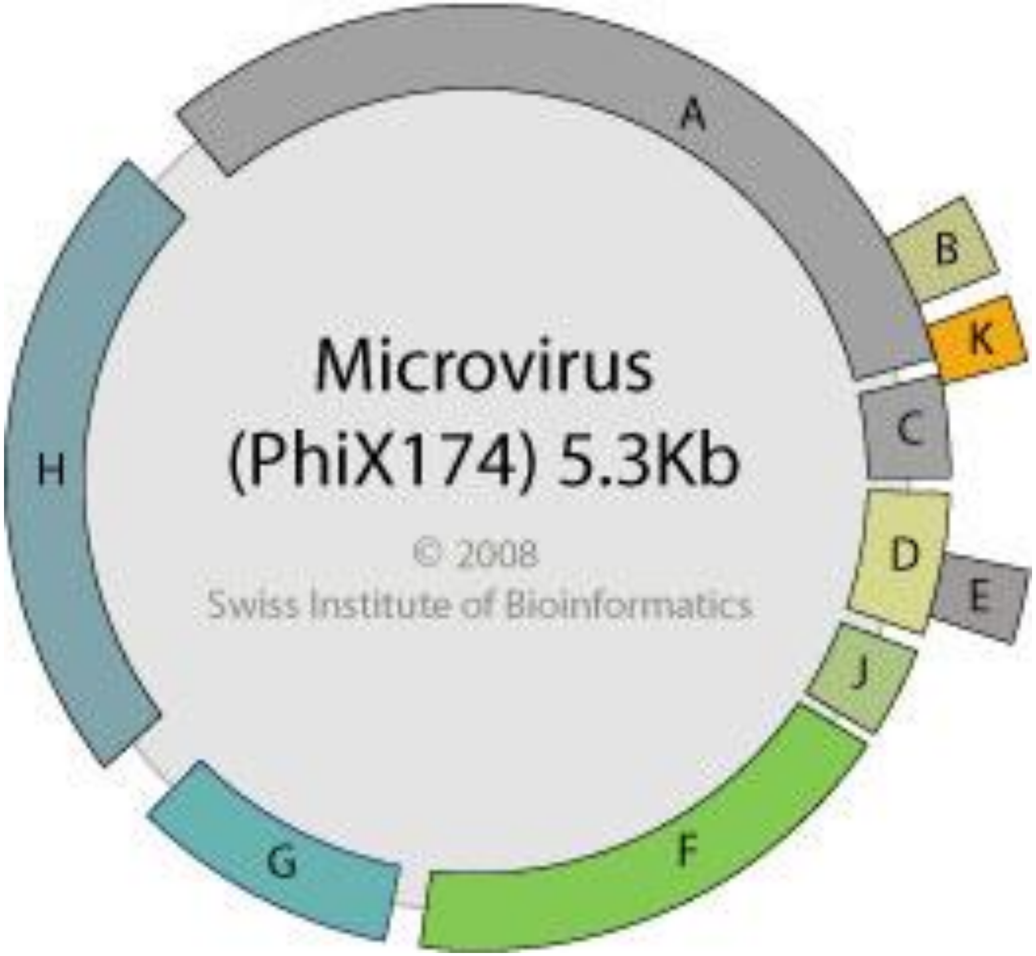




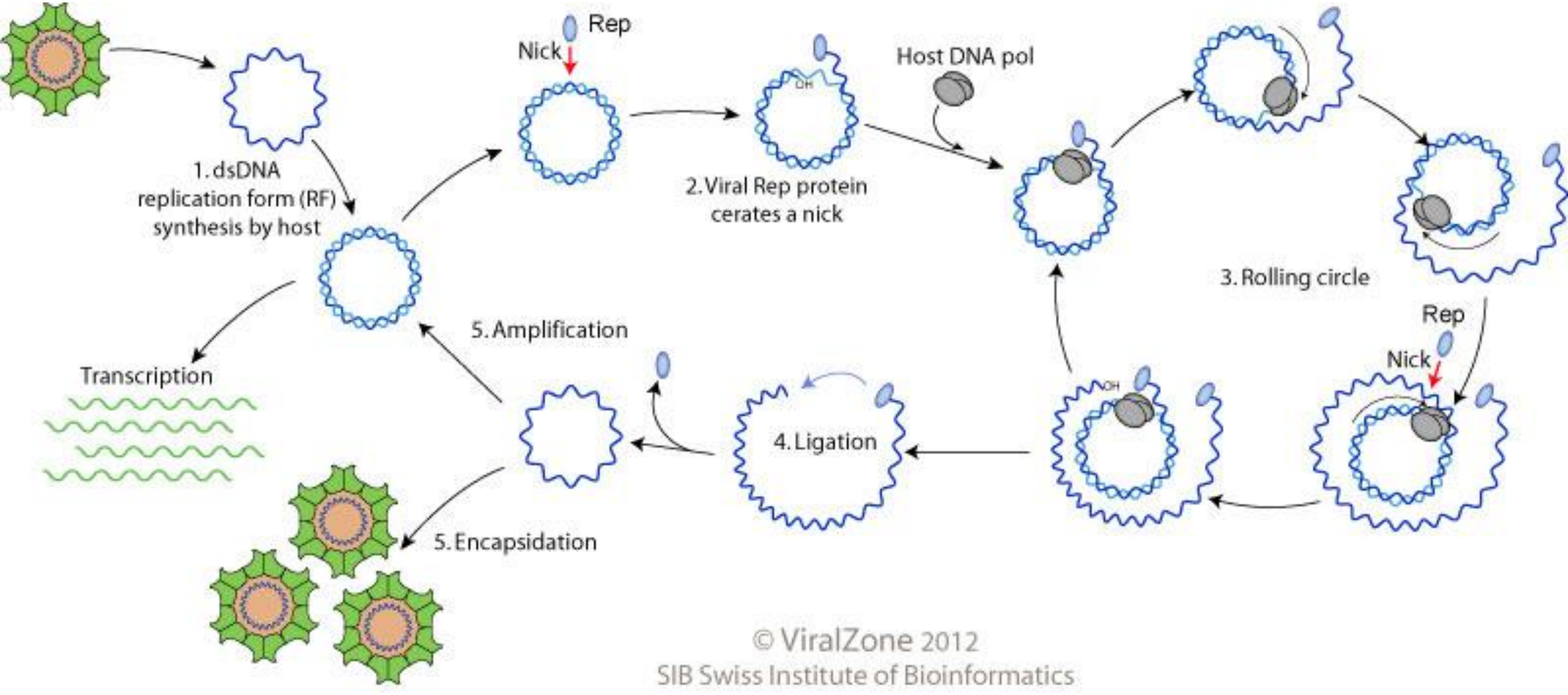


phiX174 genome

ssDNA(+) genome of 4.4 to 6.1kb



phiX174 rolling circle genome replication



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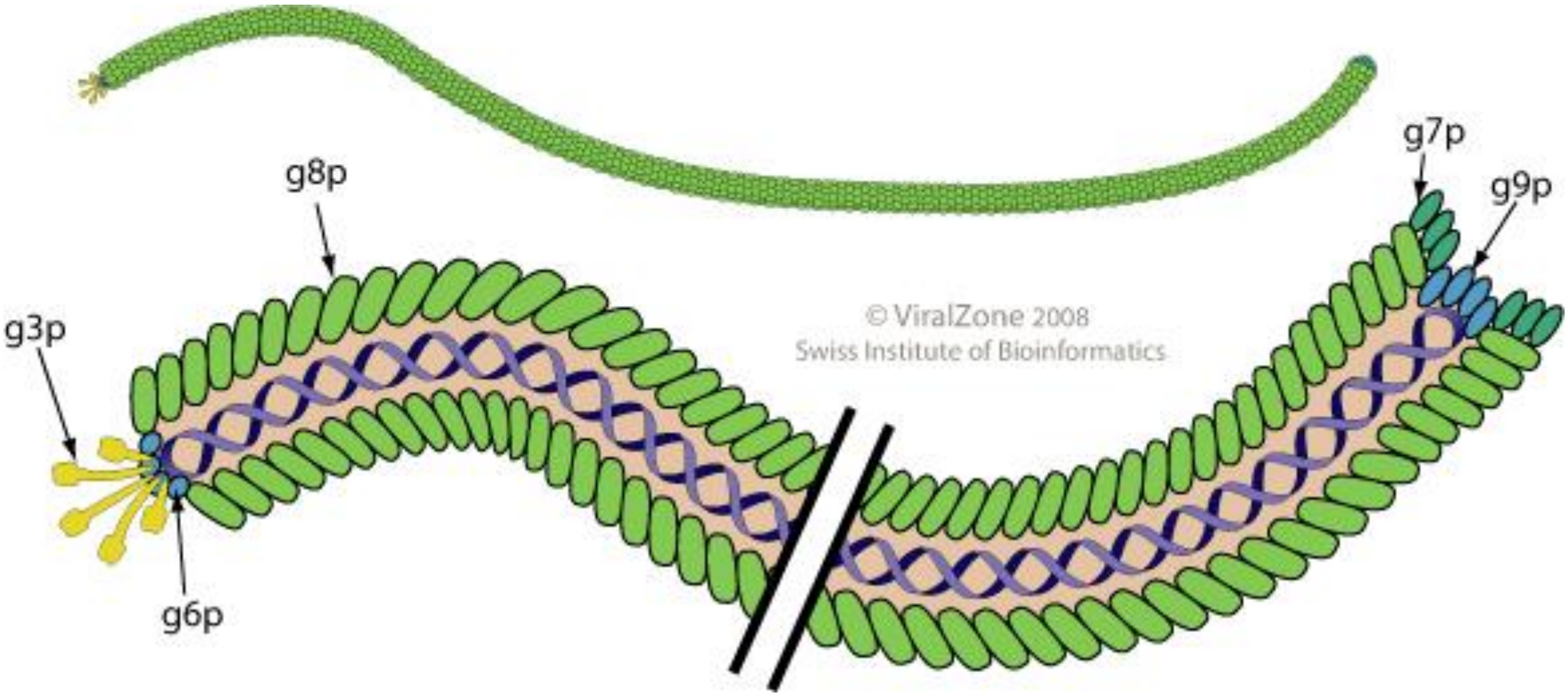
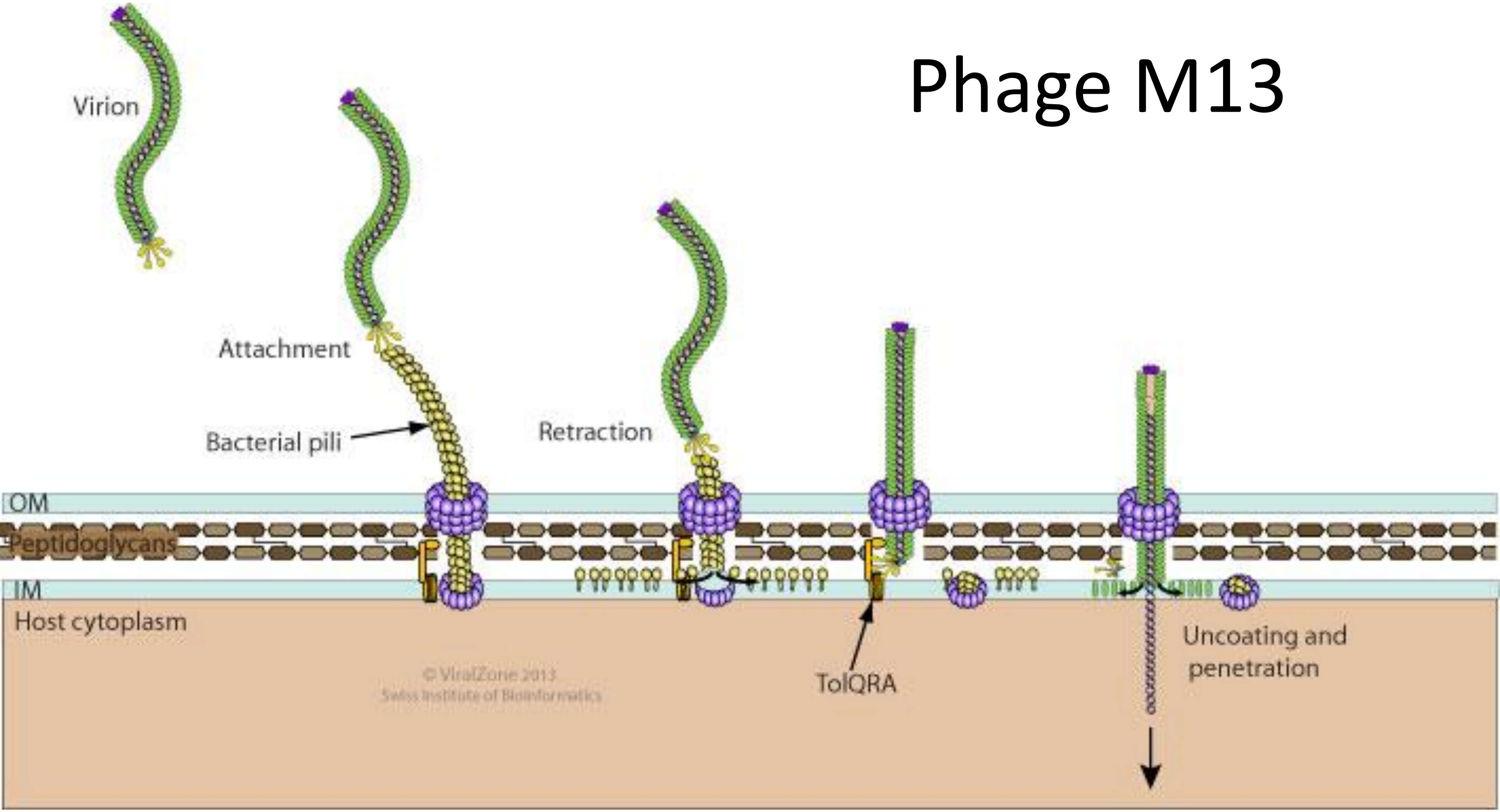
Innoviridae – M13



Non-enveloped, rod of filaments of 7nm in diameter and 700 to 2000nm in length. Helical capsid with adsorption proteins on one end.

Viral penetration into host cytoplasm via pilus retraction

Phage M13



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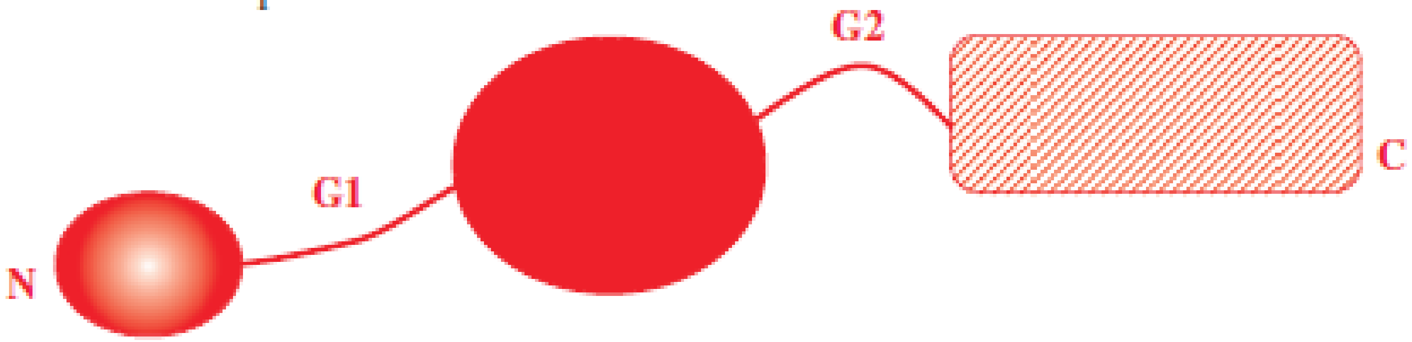
Innoviridae – gene product III

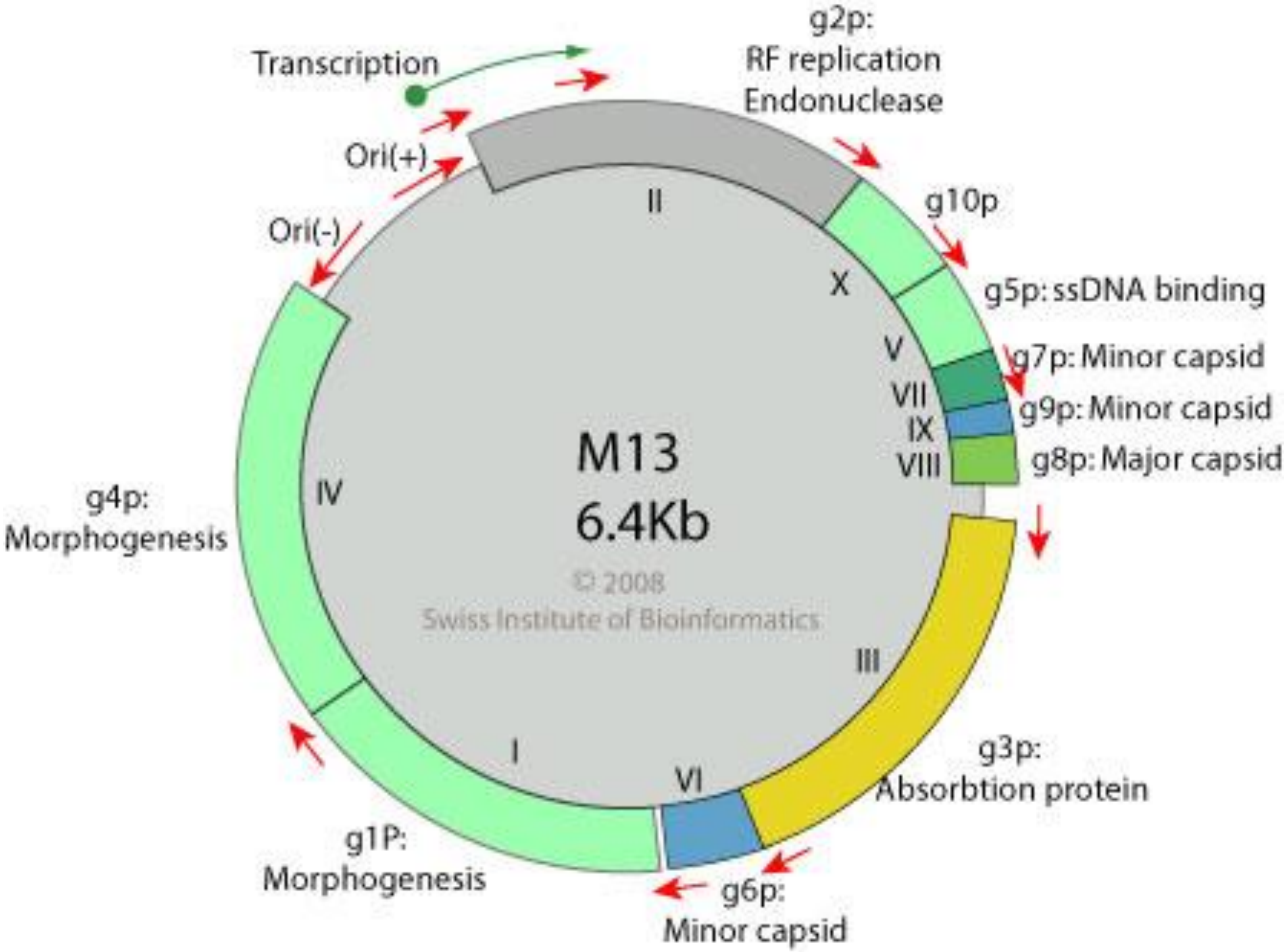


N1 Domain
Membrane penetration; interaction with TolA co-receptor

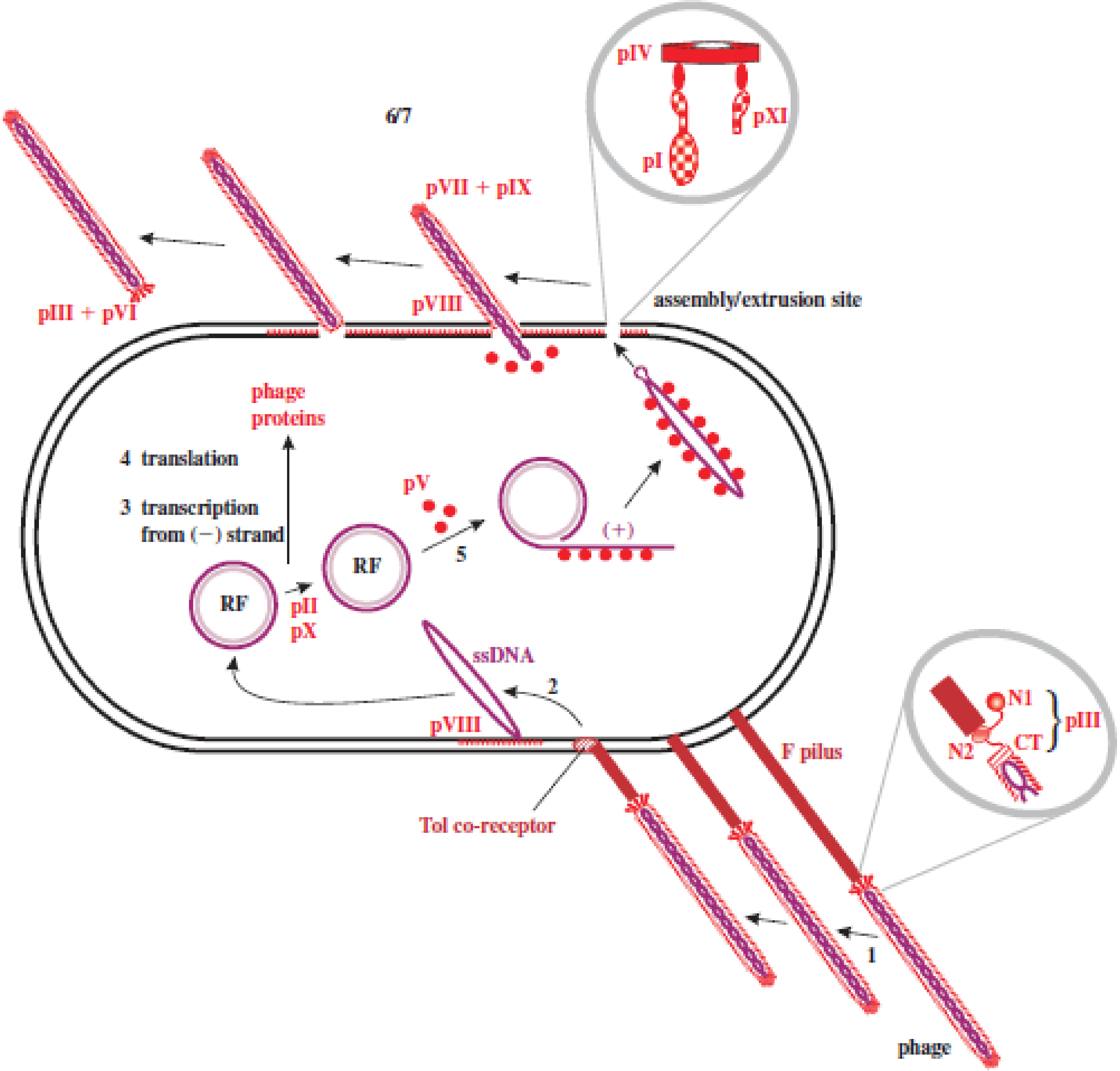
N2 Domain
Adsorption to receptor (tip of F pilus)

CT Domain
Anchor in virion

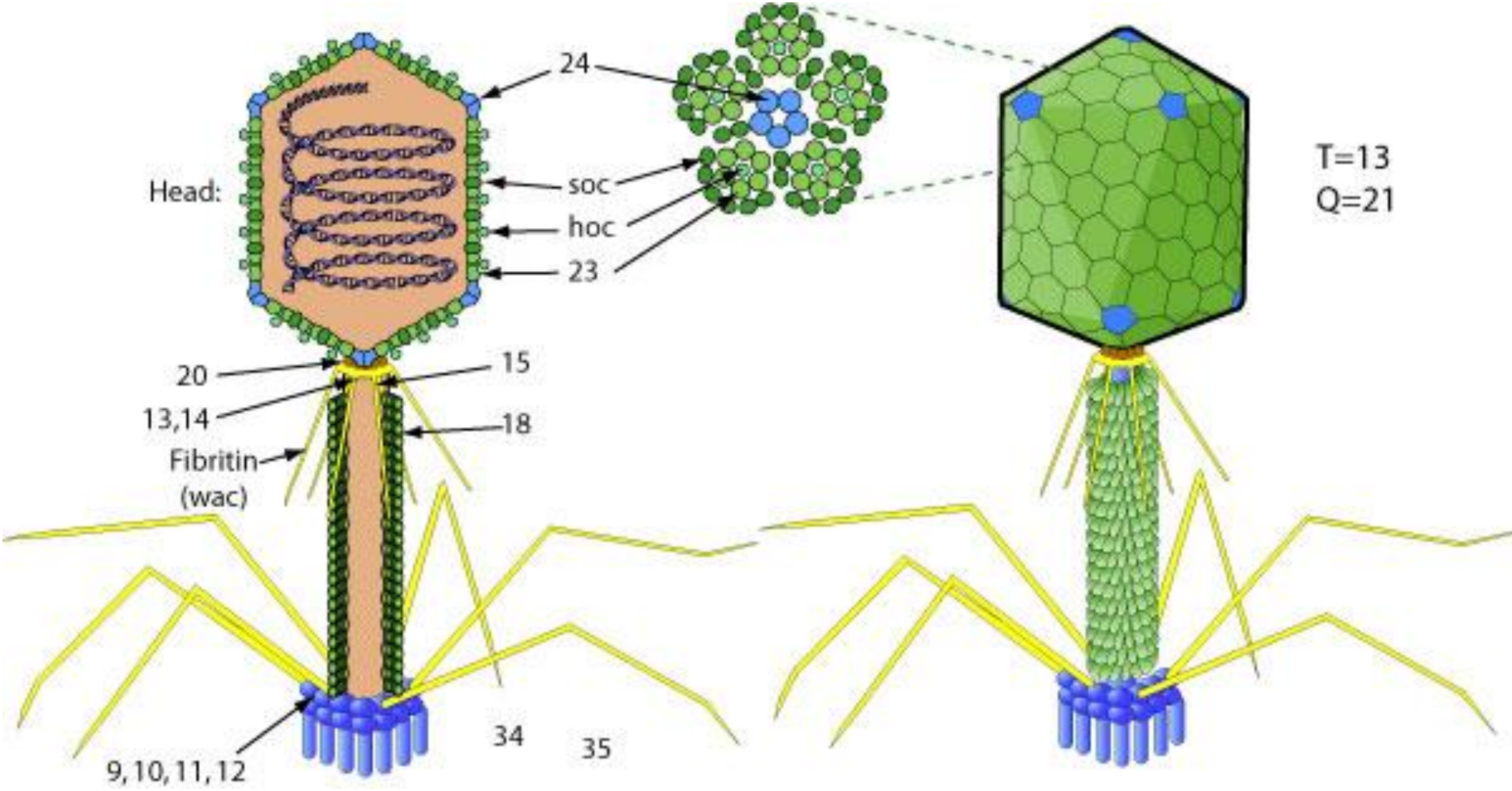
M13 genome (rolling circle replication)

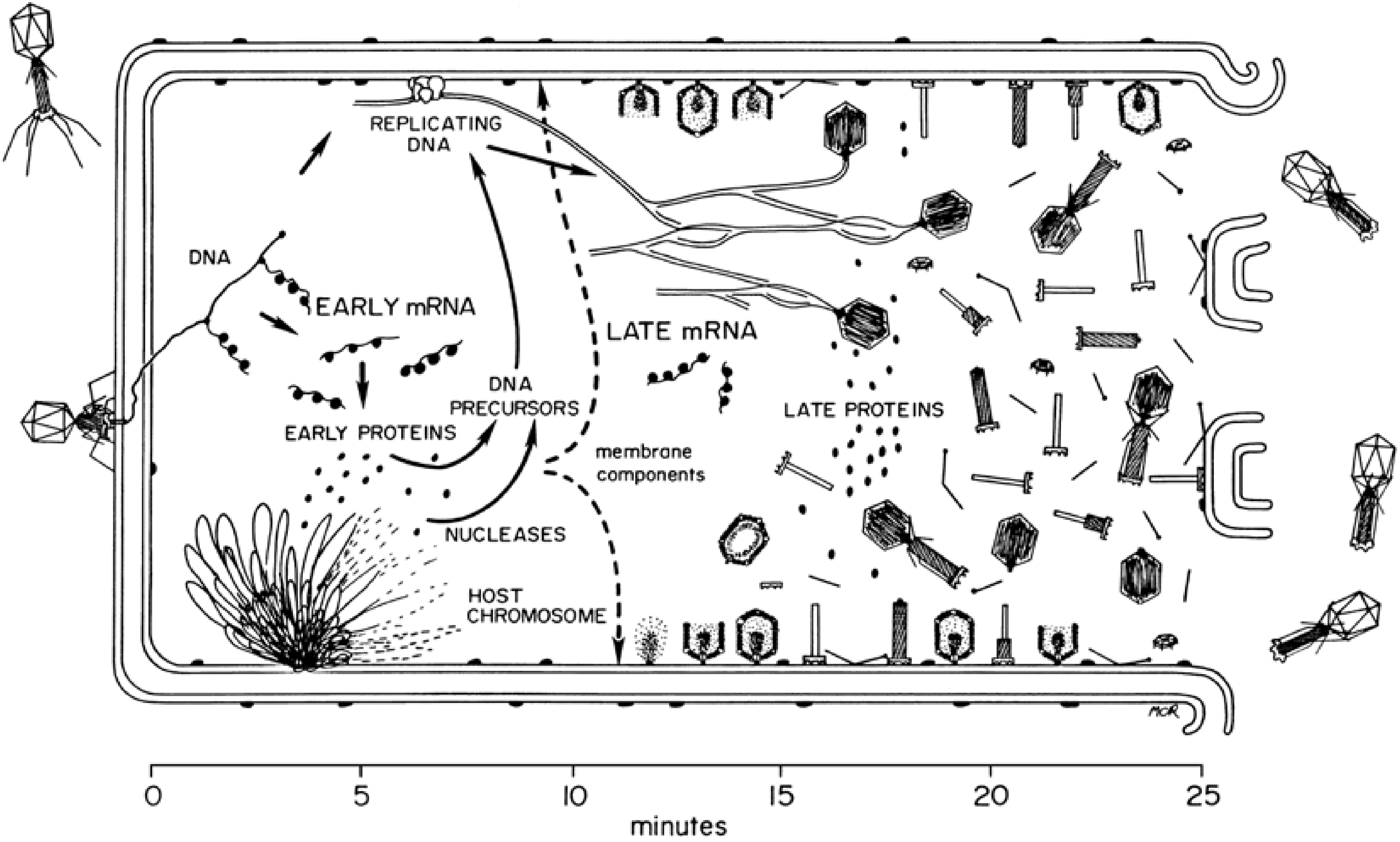


6/7

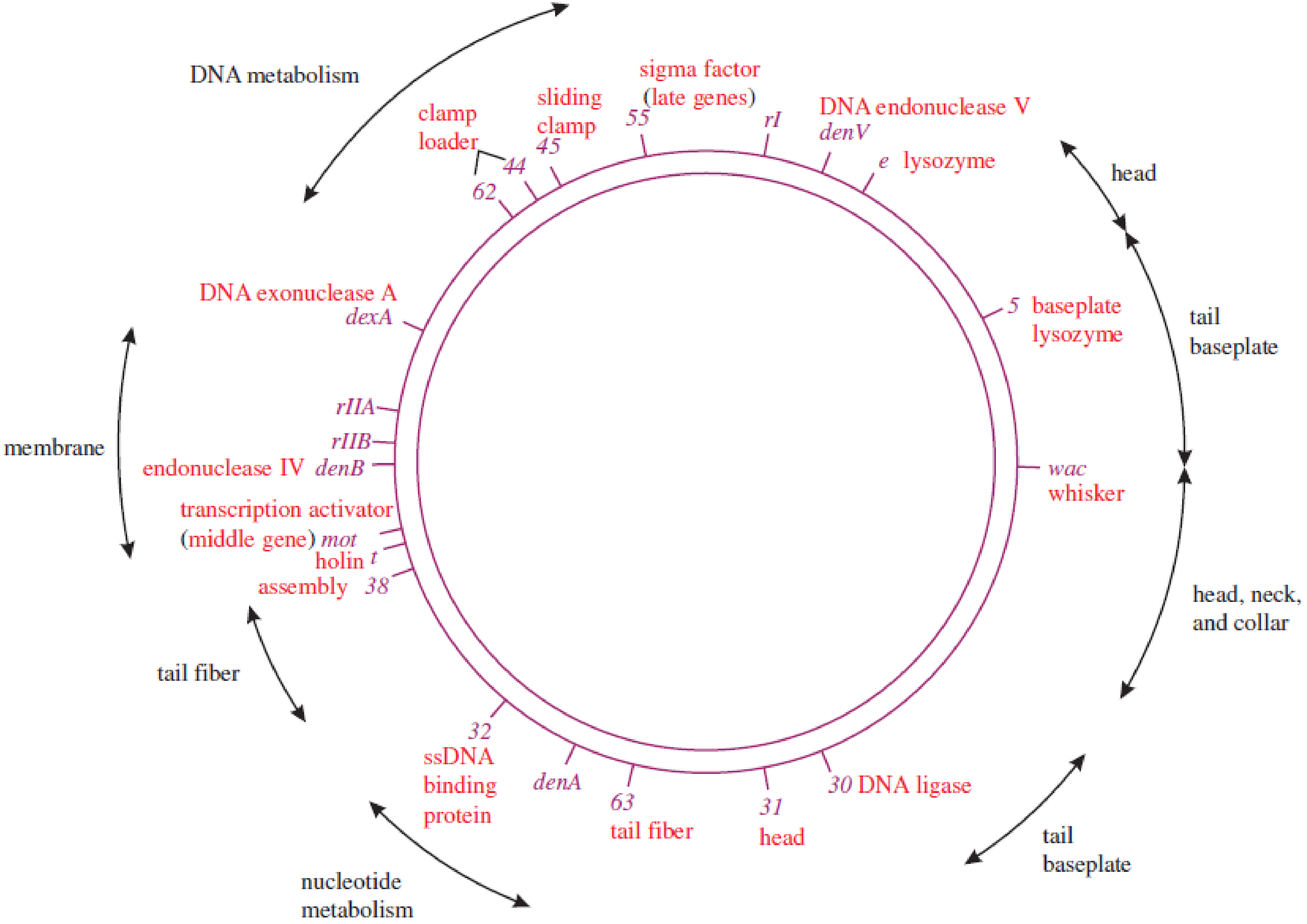

pIV
pXI
pI
pVII + pIX
pIII + pVI
pVIII
assembly/extrusion site
phage
proteins
4 translation
3 transcription
from (−) strand
pV
(+)
5
RF
RF
pII
pX
ssDNA
2
pVIII
N1
N2
CT
pIII
F pilus
Tol co-receptor
1
phage

Myoviridae – T4

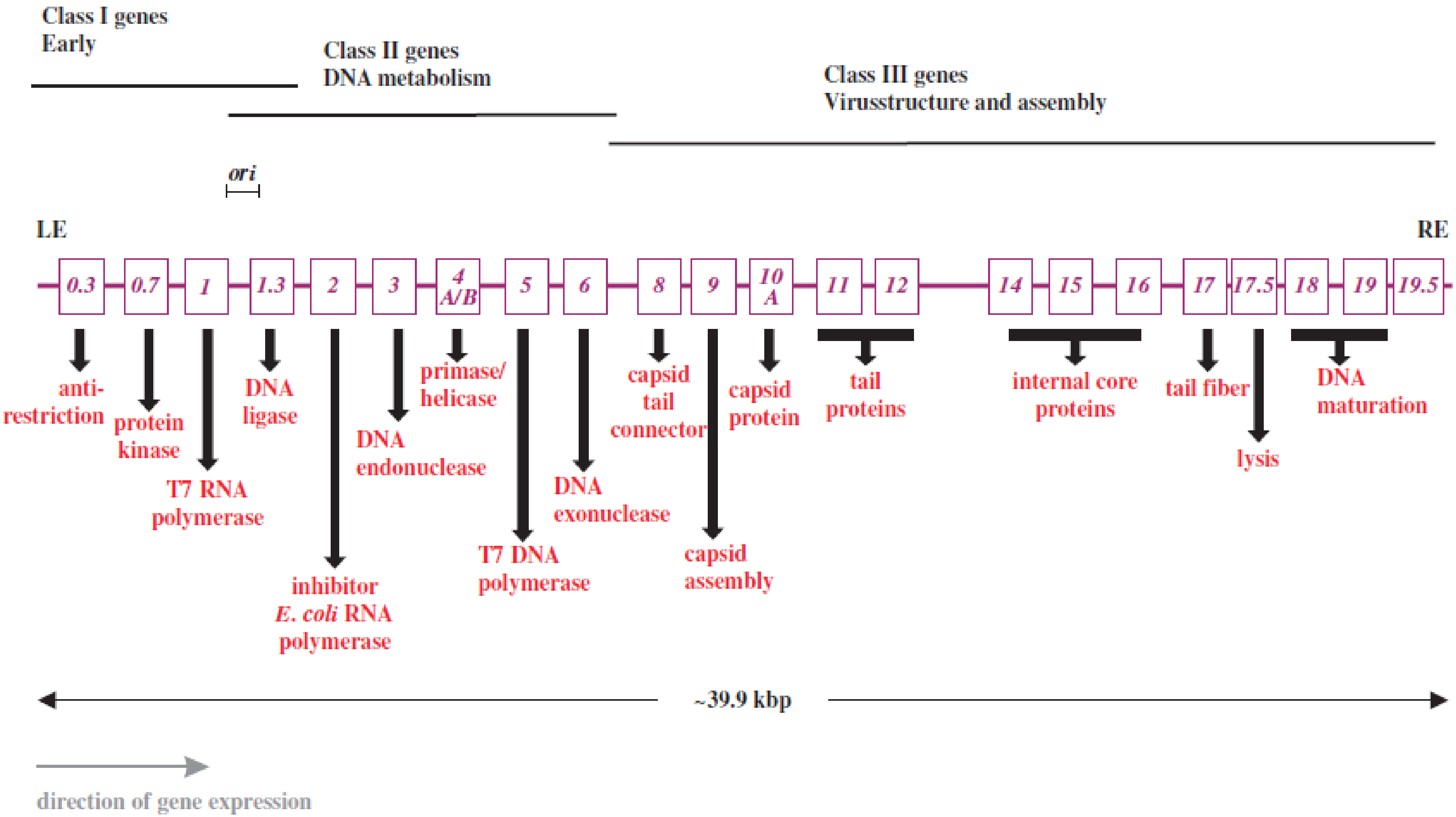



REPLICATING DNA
DNA
EARLY mRNA
LATE mRNA
DNA PRECURSORS
EARLY PROTEINS
LATE PROTEINS
membrane components
NUCLEASES
HOST CHROMOSOME
MCR
0
5
10
15
20
25
minutes

T4 – genome


DNA metabolism
clamp loader
62
44
sliding clamp
45
sigma factor (late genes)
55
rI
DNA endonuclease V
denV
e lysozyme
head
5 baseplate lysozyme
tail baseplate
DNA exonuclease A
dexA
rIIA
rIIB
membrane
endonuclease IV denB
wac whisker
transcription activator (middle gene) mot
holin t
assembly 38
head, neck, and collar
tail fiber
32
ssDNA binding protein
denA
63
tail fiber
31
head
30 DNA ligase
tail baseplate
nucleotide metabolism

Podoviridae – phage T7



Class I genes
Early

Class II genes
DNA metabolism

Class III genes
Virusstructure and assembly

ori

LE

RE

0.3 *0.7* *1* *1.3* *2* *3* *4 A/B* *5* *6* *8* *9* *10 A* *11* *12* *14* *15* *16* *17* *17.5* *18* *19* *19.5*

anti-restriction

protein kinase

T7 RNA polymerase

DNA ligase

inhibitor *E. coli* RNA polymerase

DNA endonuclease

primase/helicase

T7 DNA polymerase

DNA exonuclease

capsid tail connector

capsid assembly

capsid protein

tail proteins

internal core proteins

tail fiber

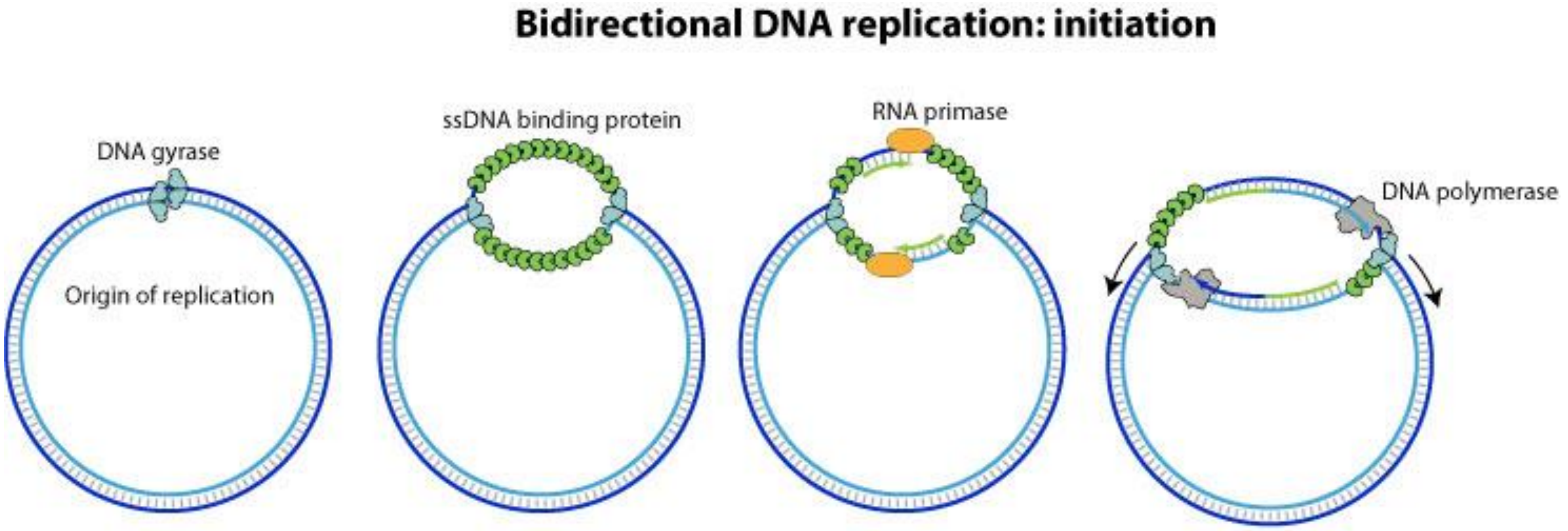
lysis

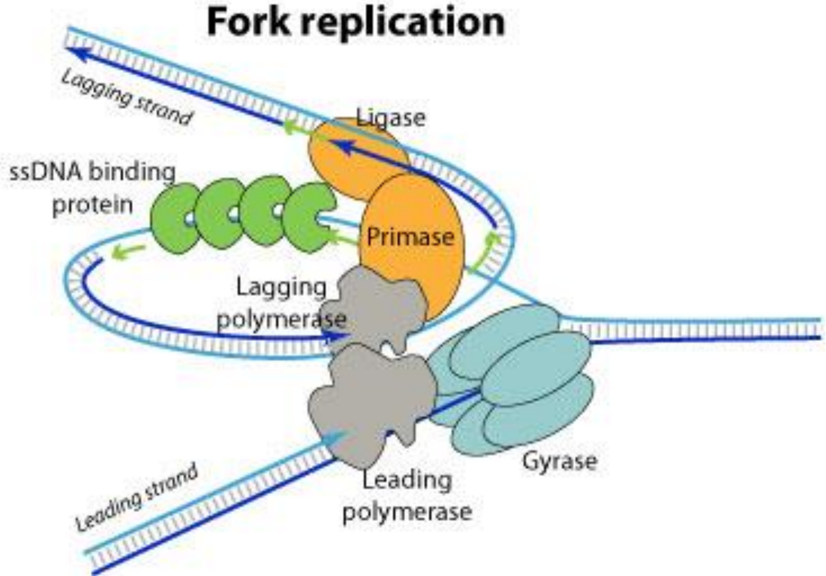
DNA maturation

~39.9 kbp

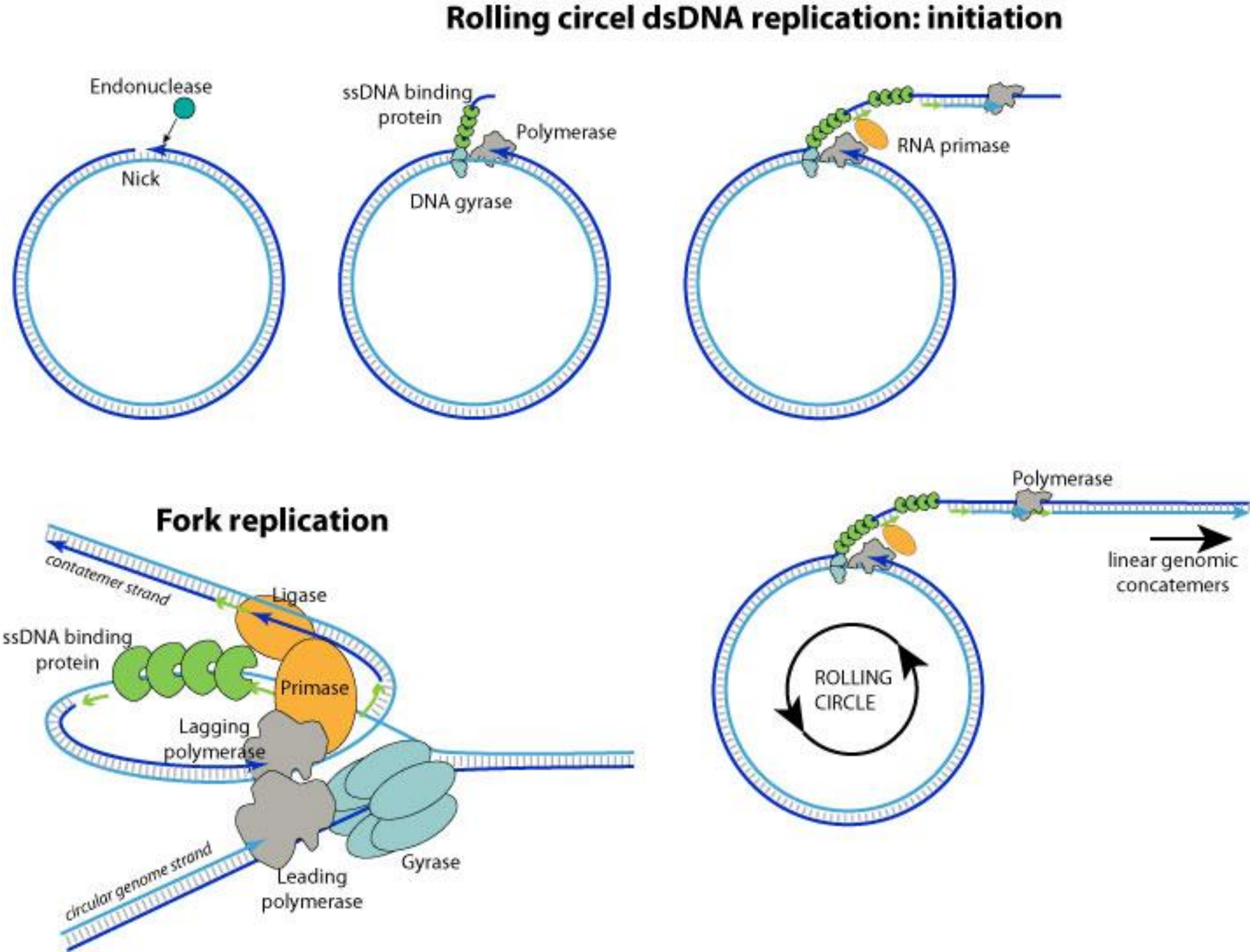
direction of gene expression

Siphoviridae – theta replication


Bidirectional DNA replication: initiation


Fork replication

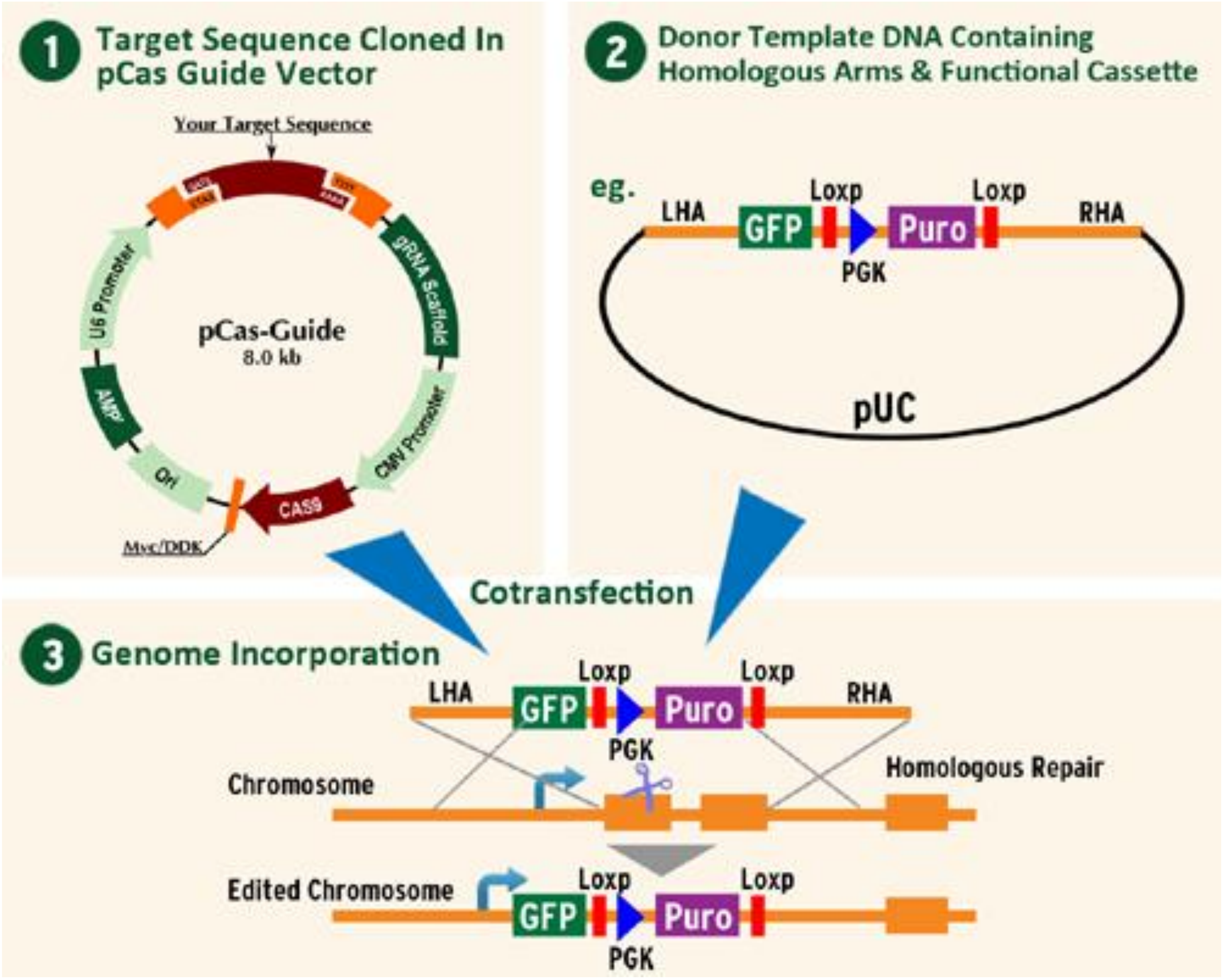
Siphoviridae – rolling circle replication



Learning outcomes

- discuss the replication cycle and control of gene expression in ssRNA coliphages
- outline the infection process of dsRNA phages
- review the biology of the filamentous and icosahedral ssDNA phages
- describe the structure and replication cycle of dsDNA phages

CRISPR-cas genome editing



Virus origins

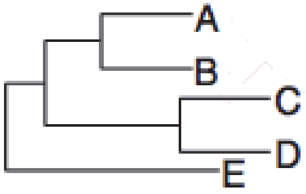
Possible Virus Origins:

- RNA molecules that existed before cells
- cell components
- micro-organisms.

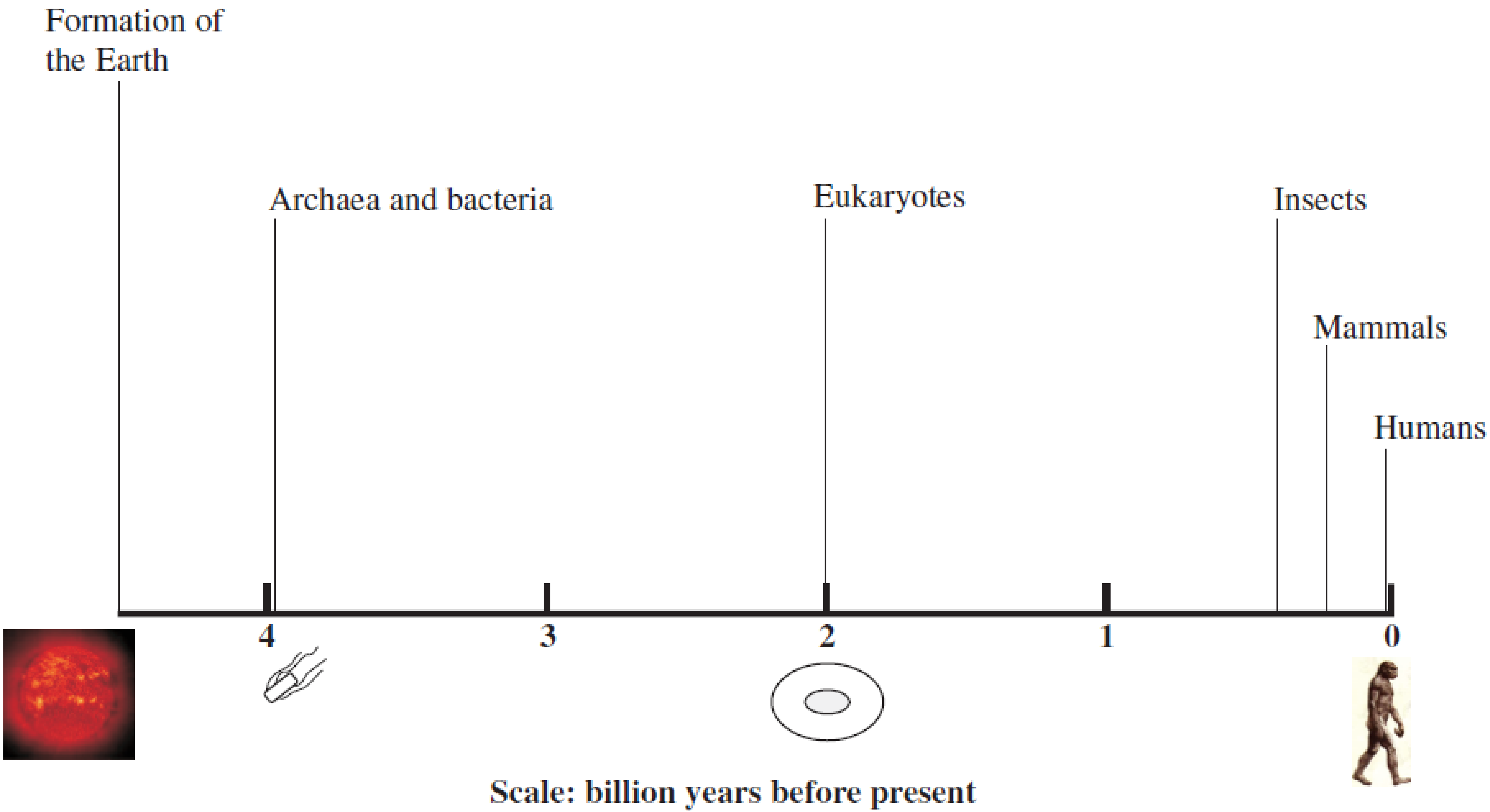
Viruses evolve as a result of:

- errors during nucleic acid replication;
- recombination between virus strains;
- reassortment between virus strains;
- acquisition of cell genes.

Evolution of viruses can be monitored by sequencing their genomes and creating phylogenetic trees:

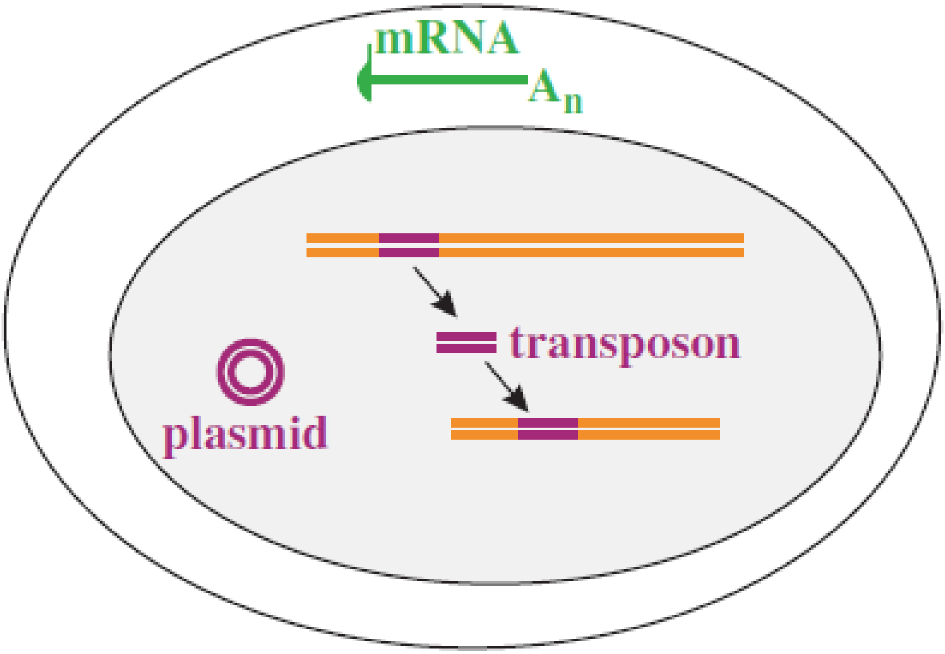


New viruses may evolve as a result of viruses infecting new host species, e.g. HIV-1 and HIV-2.

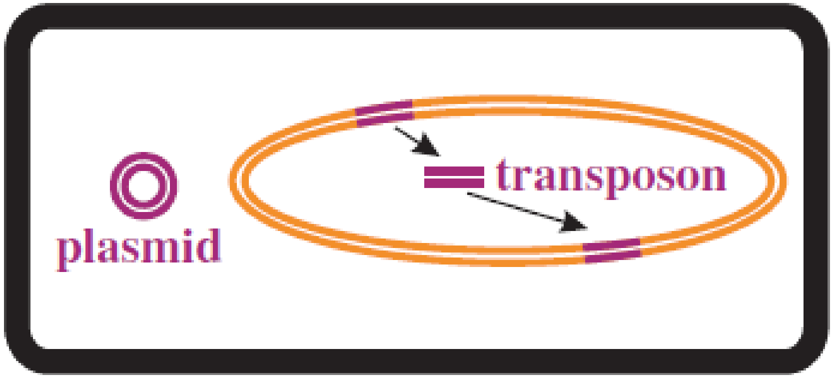

Formation of the Earth
Archaea and bacteria
Eukaryotes
Insects
Mammals
Humans
4
3
2
1
0
Scale: billion years before present

Potential virus precursors

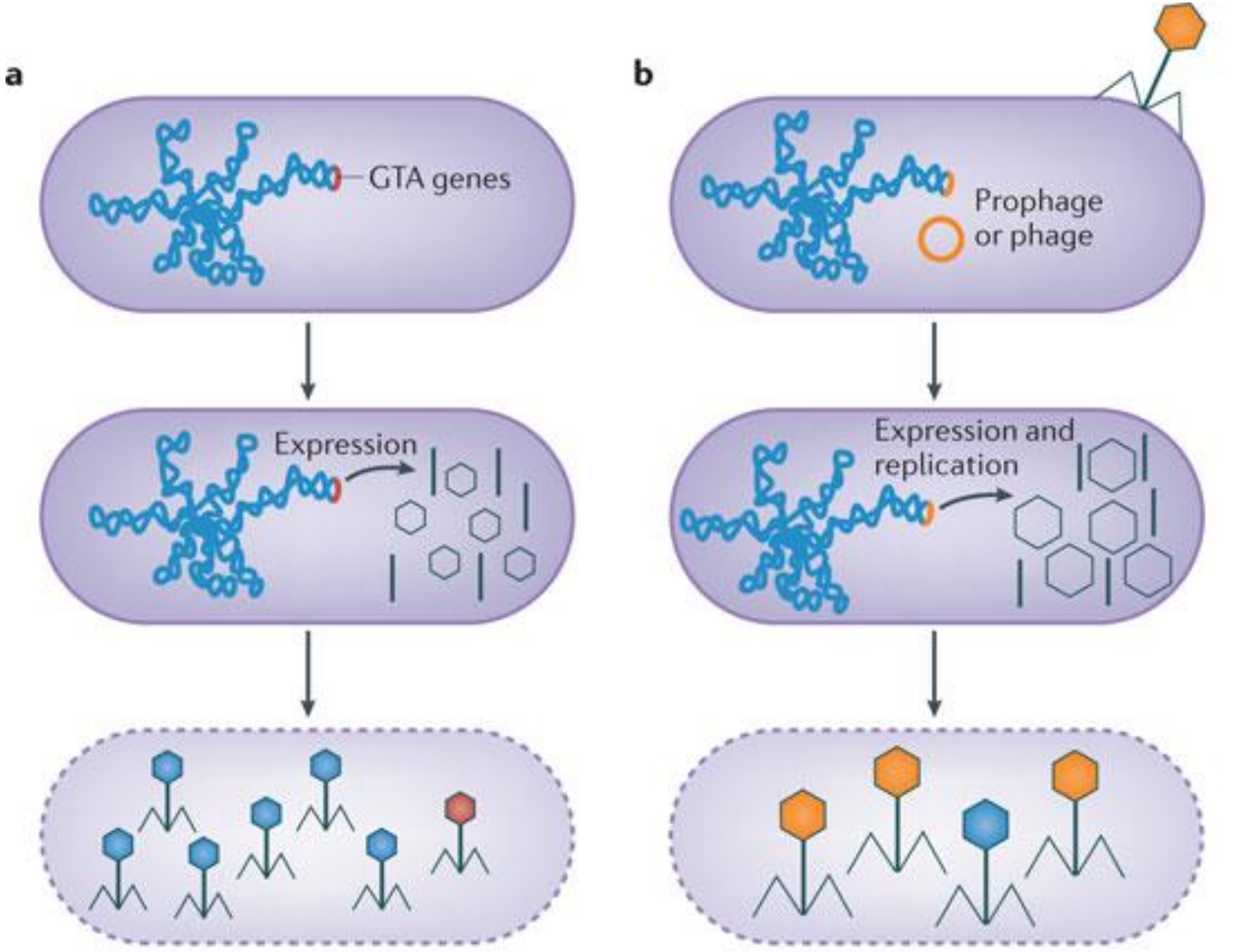
eukaryotic cell



prokaryotic cell



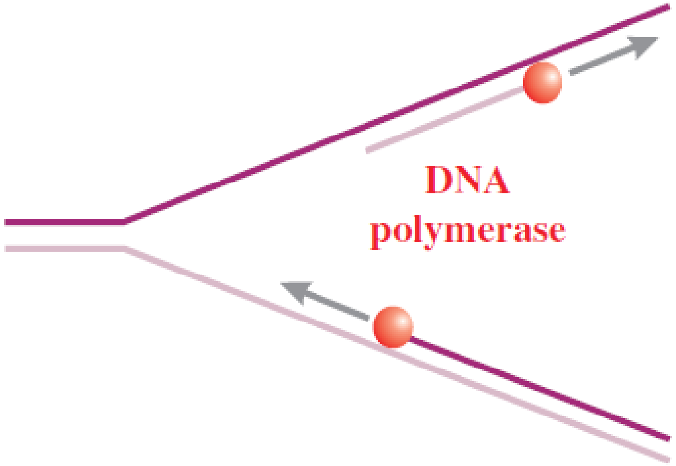
Gene transfer agents



Nature Reviews | Microbiology

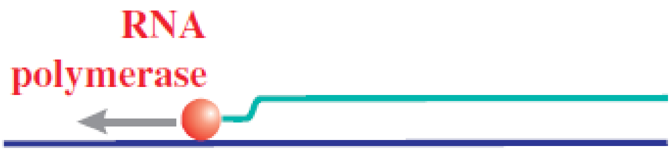
Polymerase error rates

DNA Replication



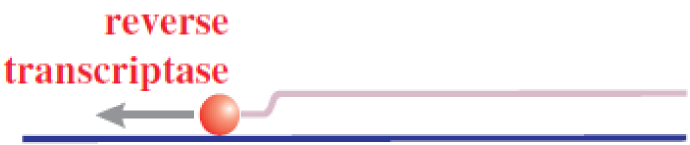
One error per
10^6 to 10^8 bases added

RNA Replication



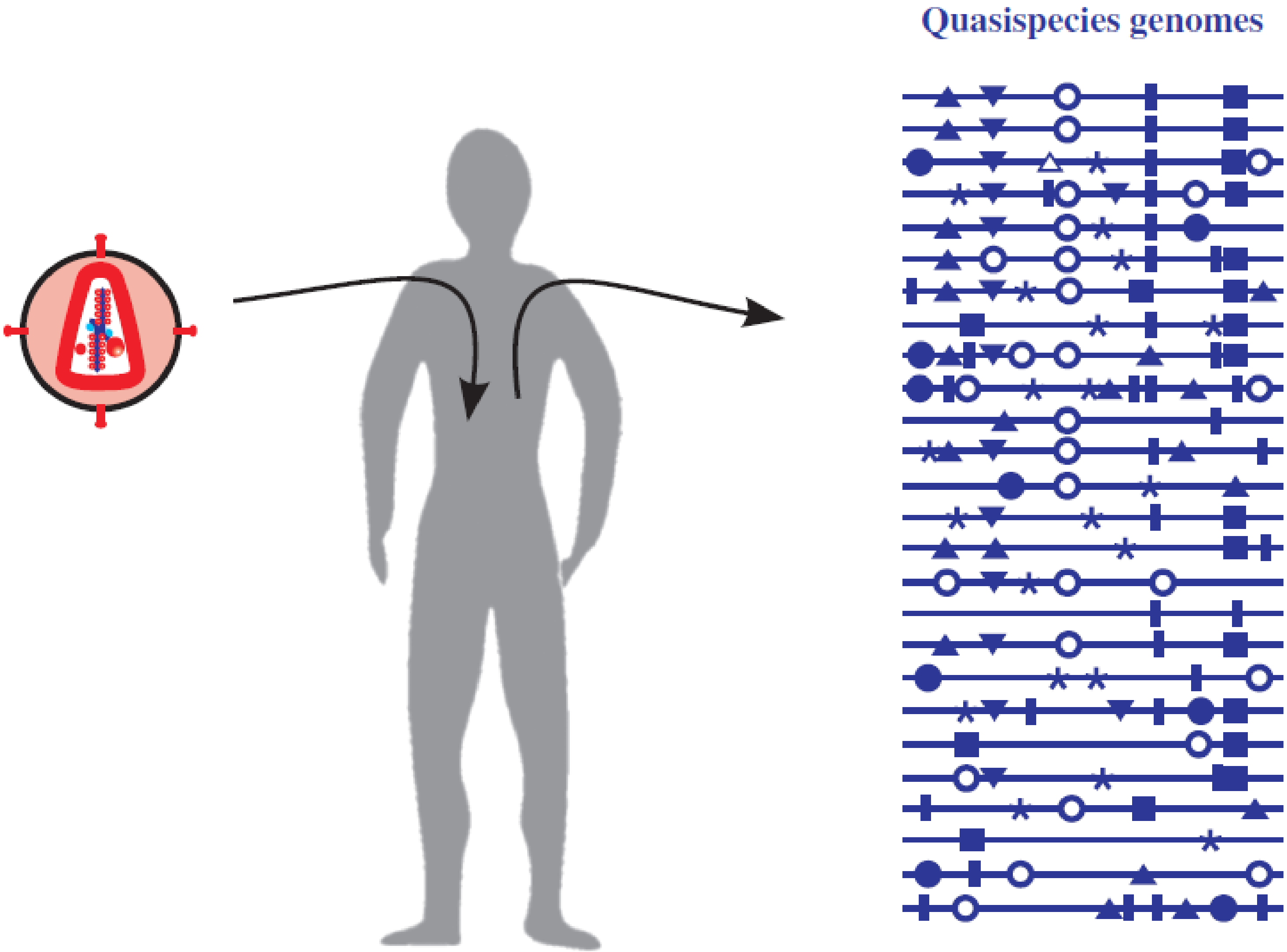
One error per
10^4 to 10^6 bases added

Reverse
Transcription

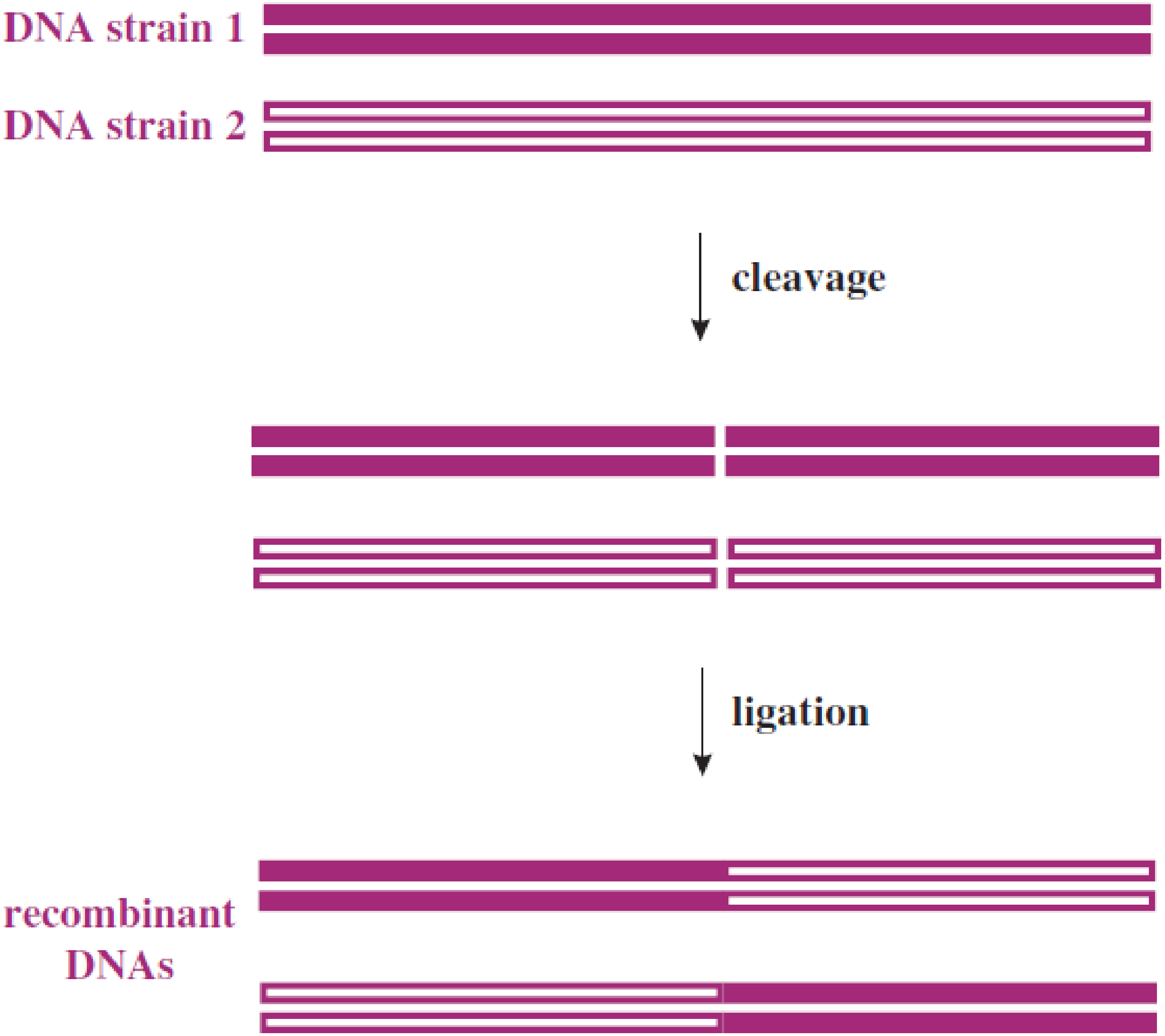


One error per
10^4 to 10^6 bases added

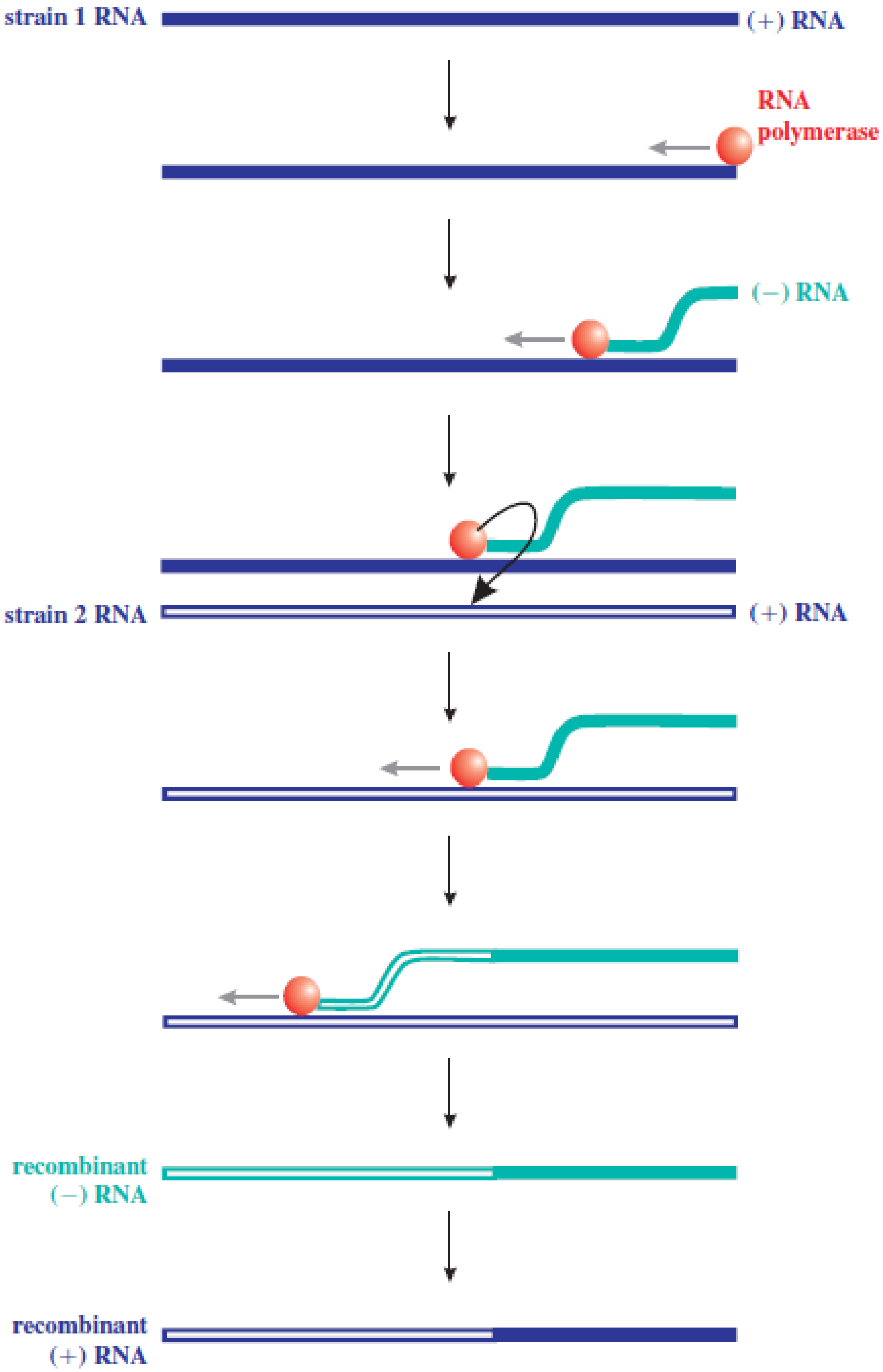
Quasispecies



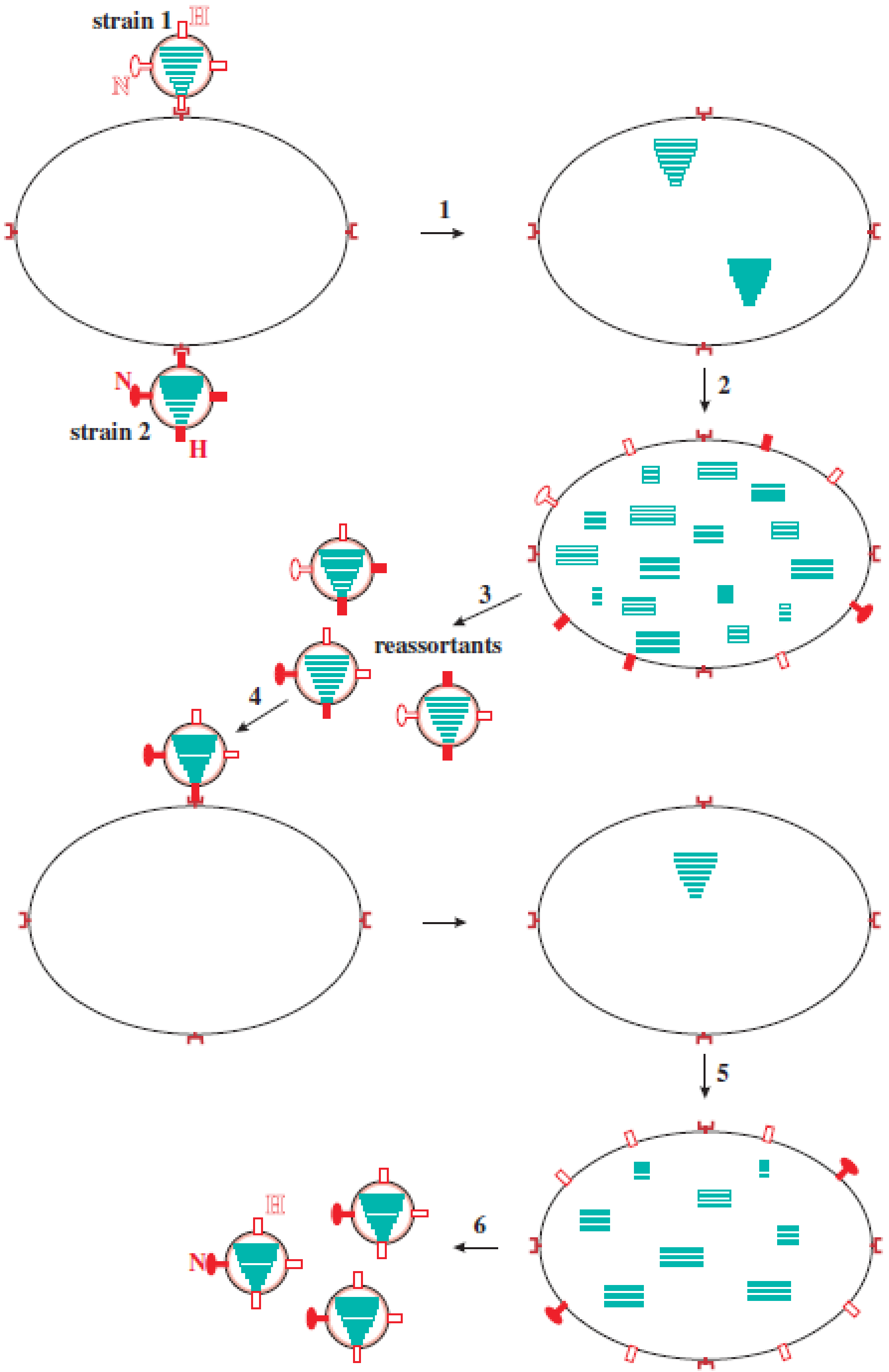
Recombination



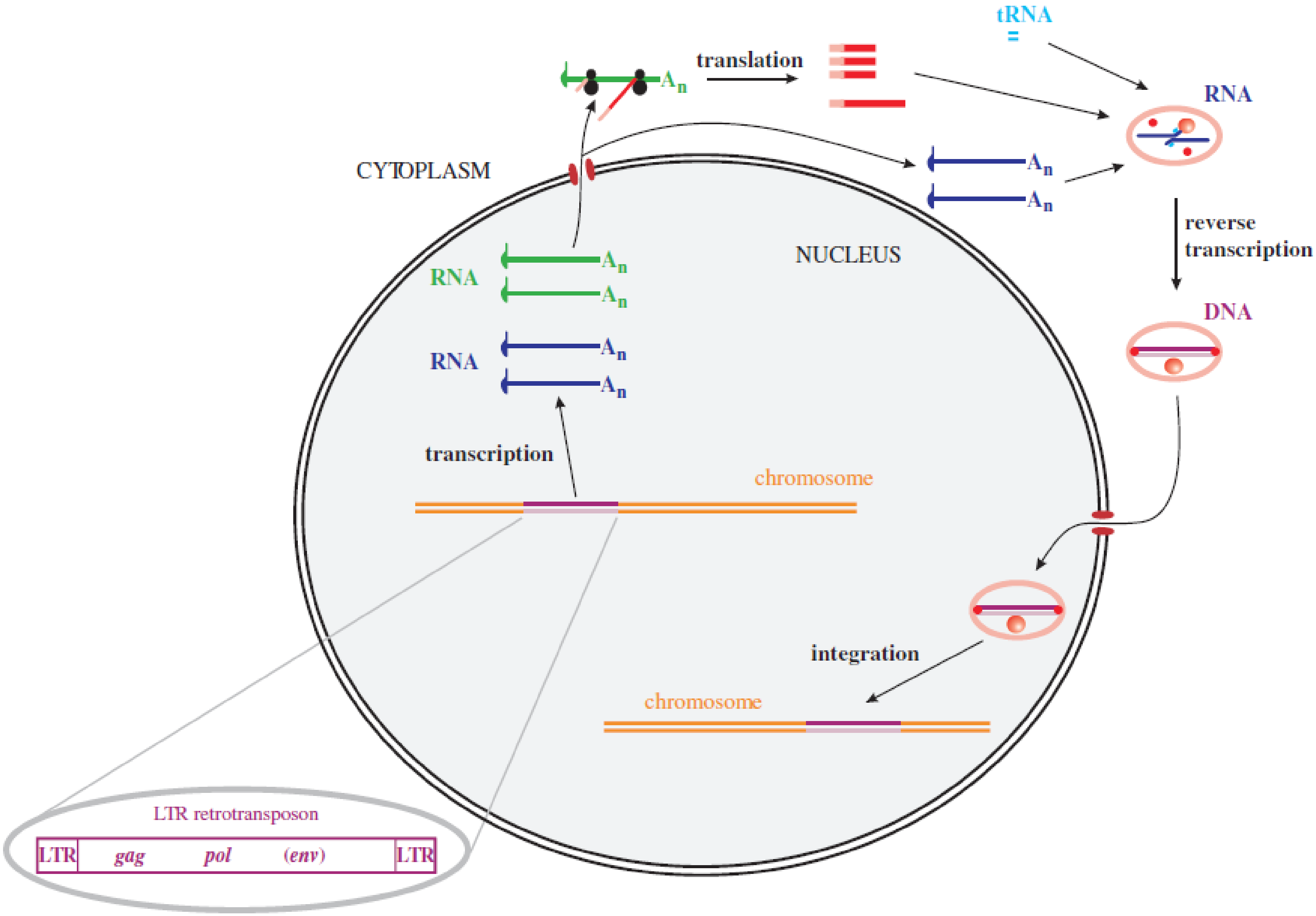
Copy-choice recombination



Genome fragment re-assortment



LTR retrotransposons



- Progressive hypothesis
- Regressive hypothesis
- Virus-first hypothesis
- Nucleocytoplasmic large DNA viruses as precursors of nuclei in eukaryotes

Learning outcomes

- evaluate theories on the origins of viruses
- explain how virus evolution occurs through mutation, recombination and re-assortment
- assess the value of virus genome sequencing in studies of virus origins and evolution
- assess the threats posed to man and animals by rapid virus evolution
- discuss the co-evolution of viruses and their hosts