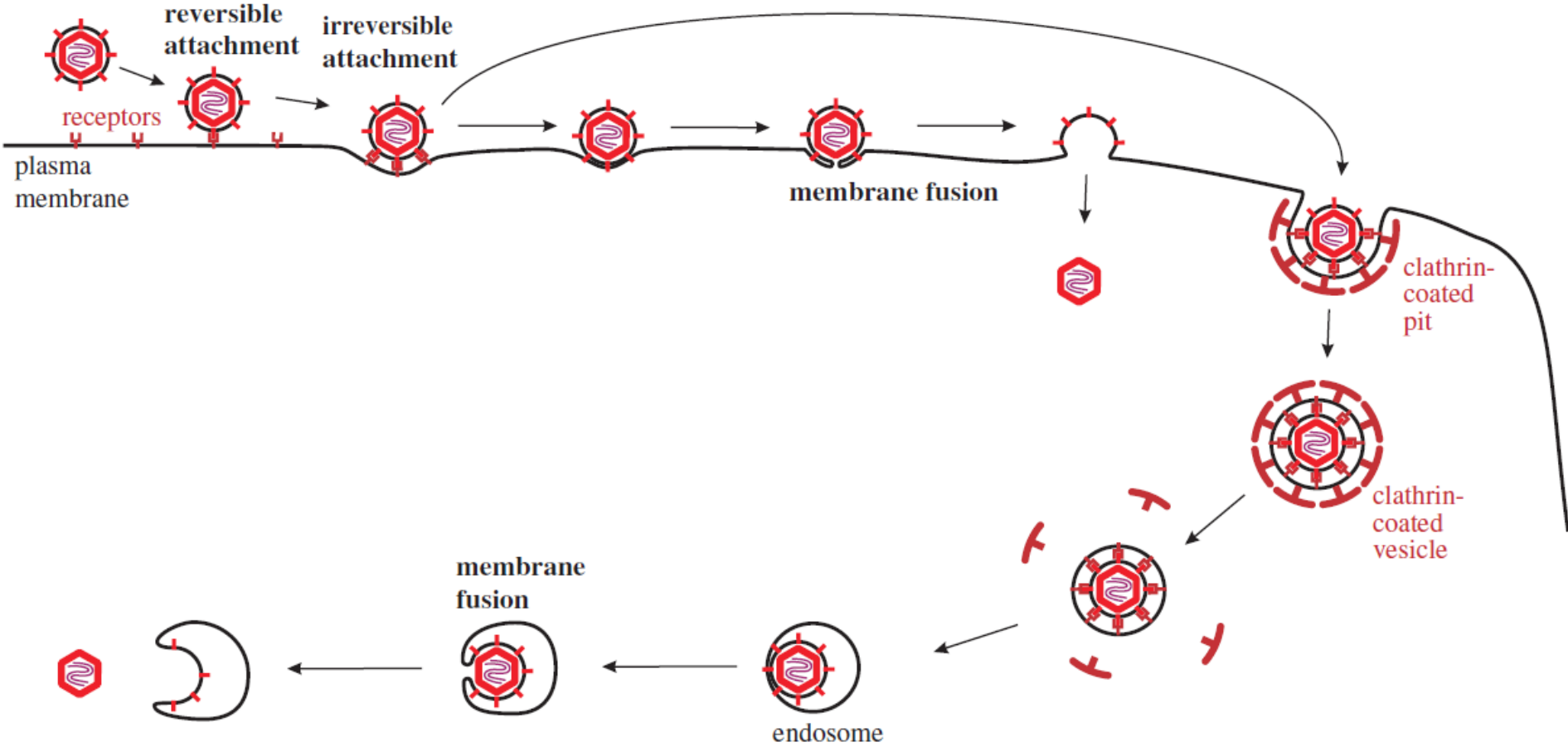


Structural Virology

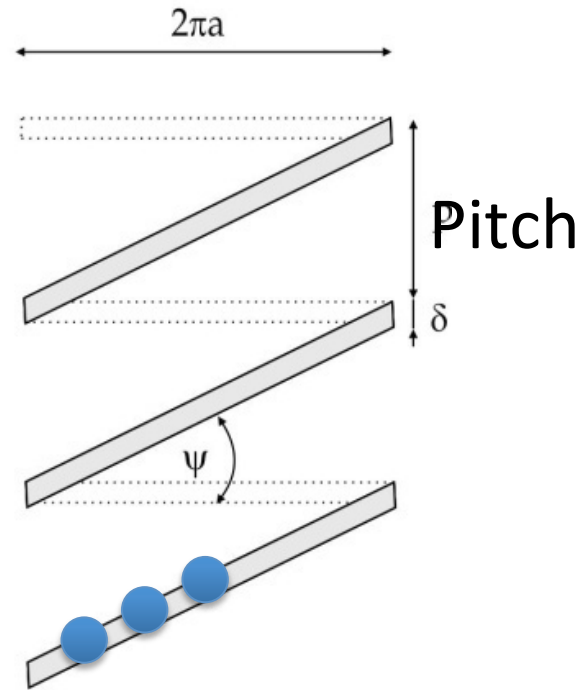
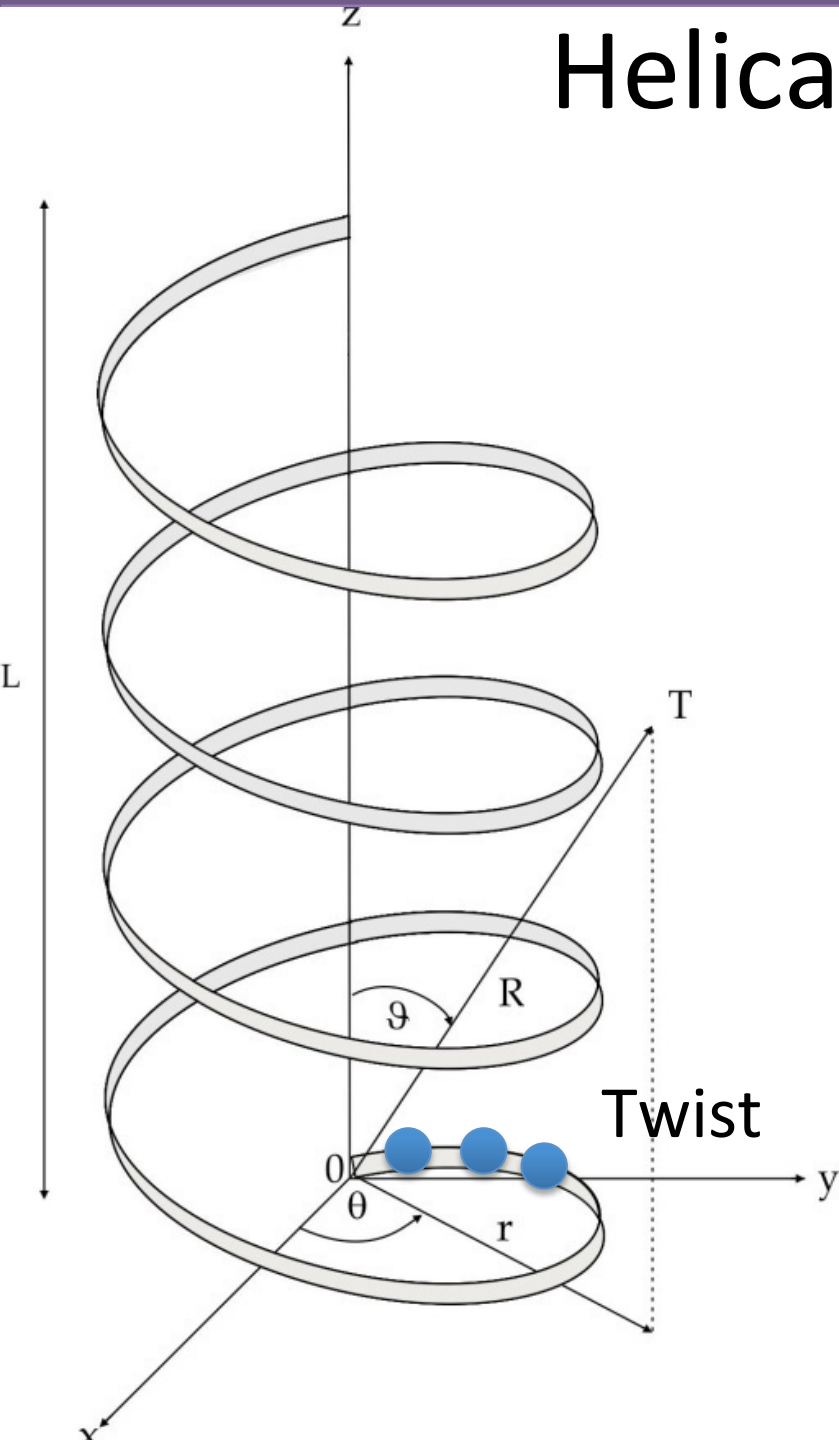
Lecture 3

Pavel Plevka

Attachment and entry of an enveloped virion

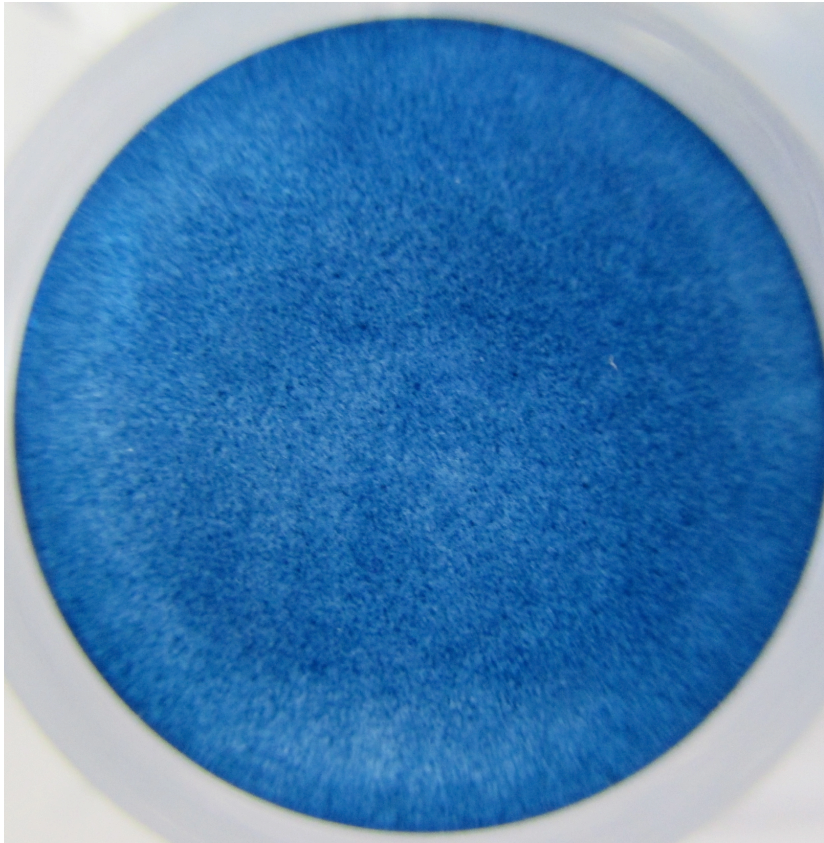


Helical symmetry

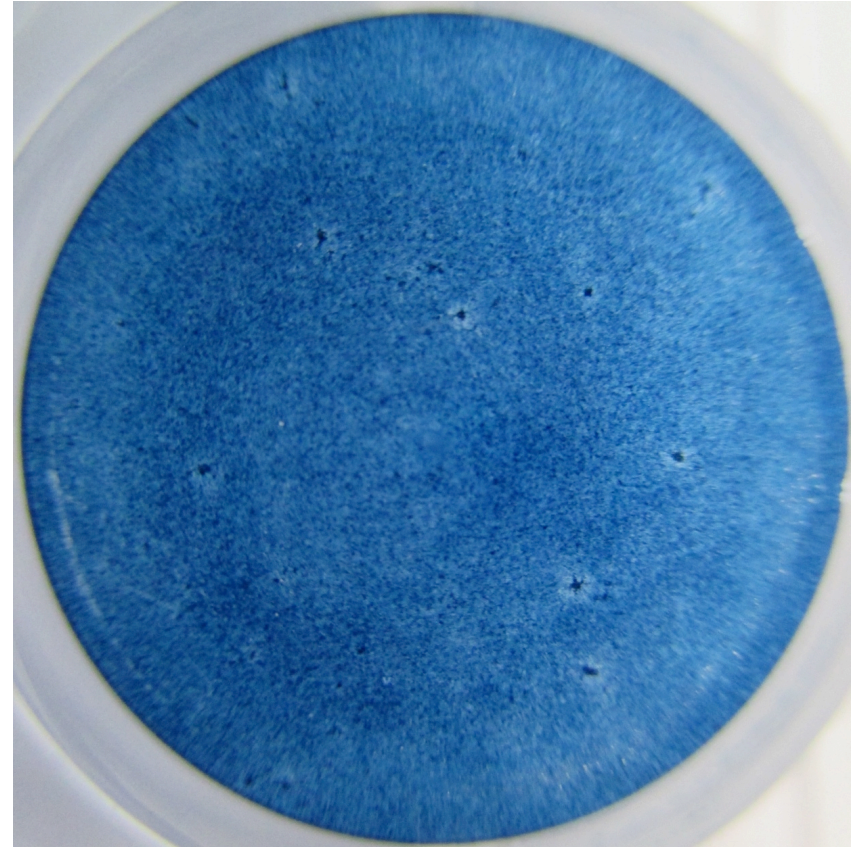


Pitch is the distance along the helix axis for one complete helix turn

Twist is the rotation between neighboring nucleotides



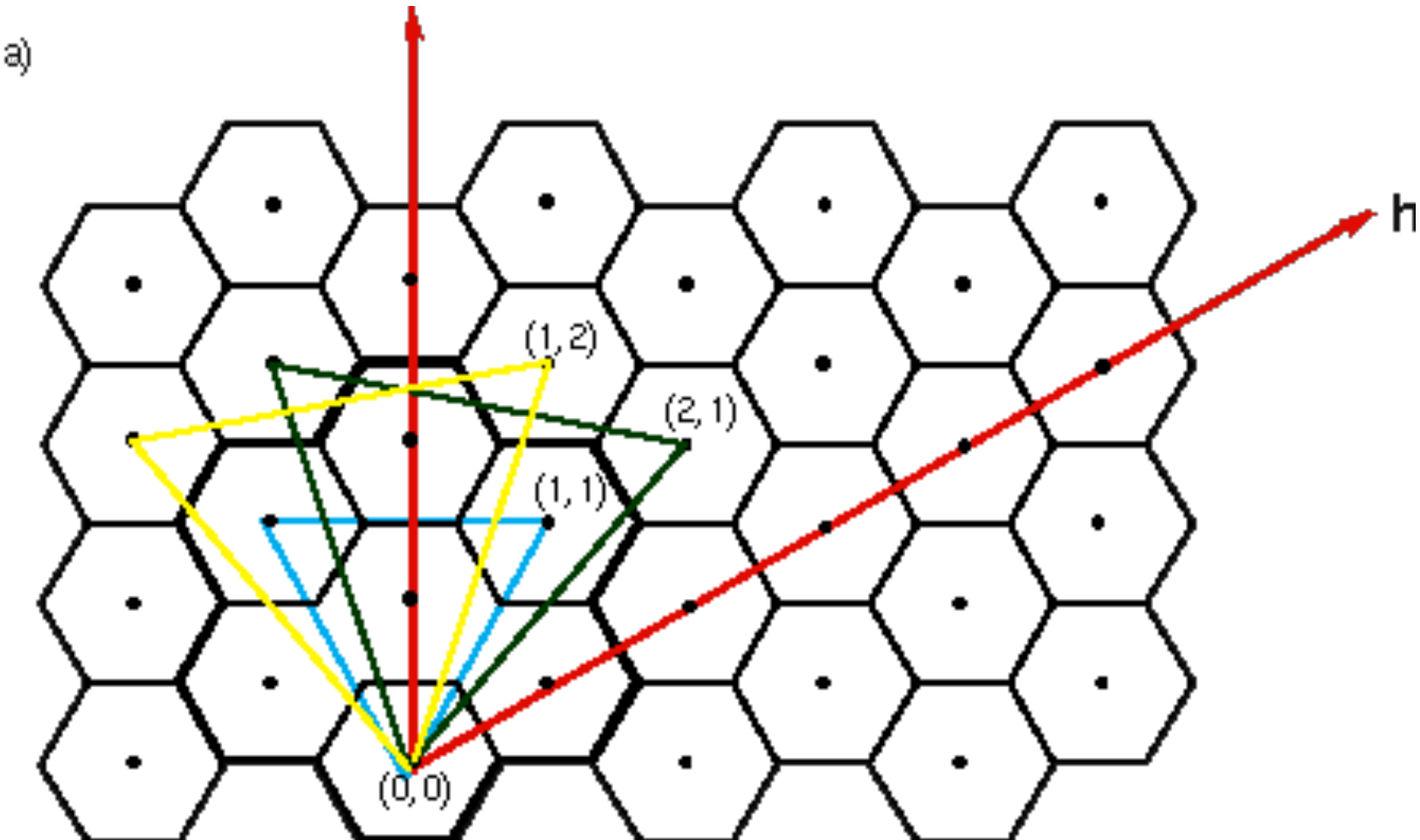
HeLa 300 tis bb./ml, 10.2.14
Fixed after 72h
BEST OF CMC



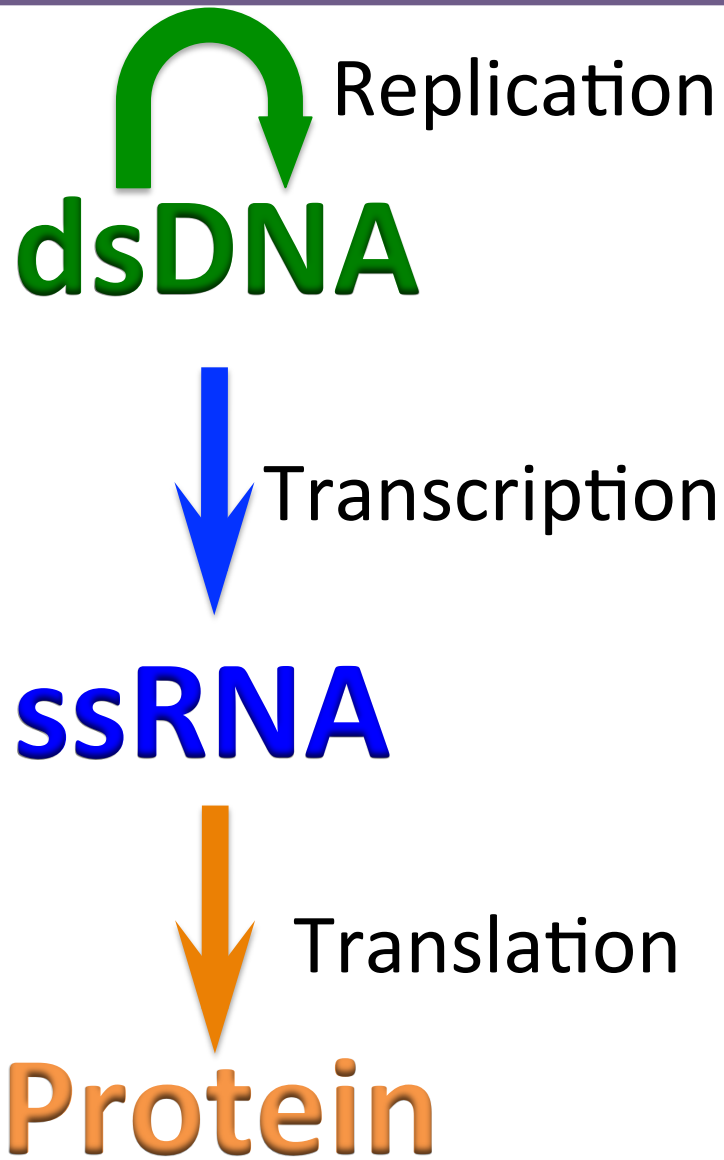
HRV16 from stock 28.11.13,
PFU $5 \cdot 10^3$

Quasi equivalence

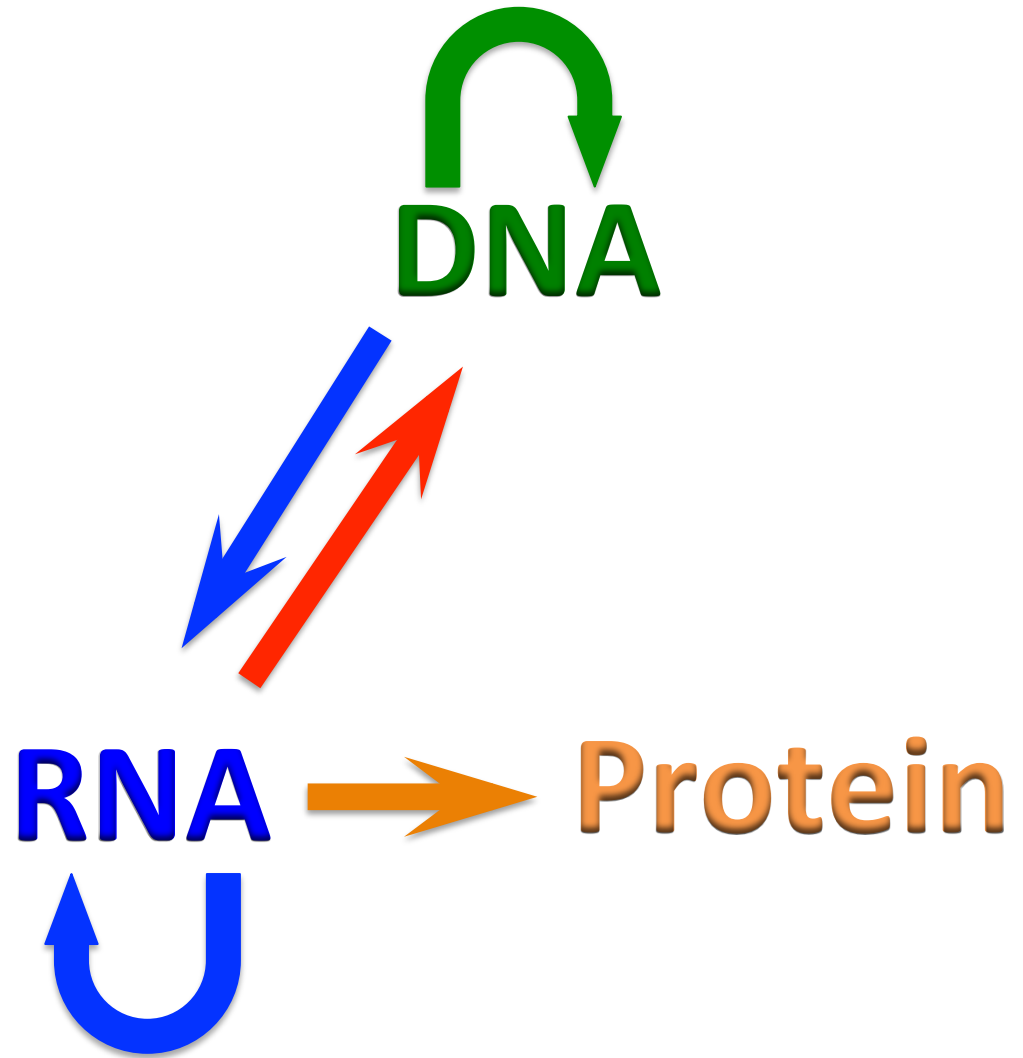
$$T = h^2 + hk + k^2$$



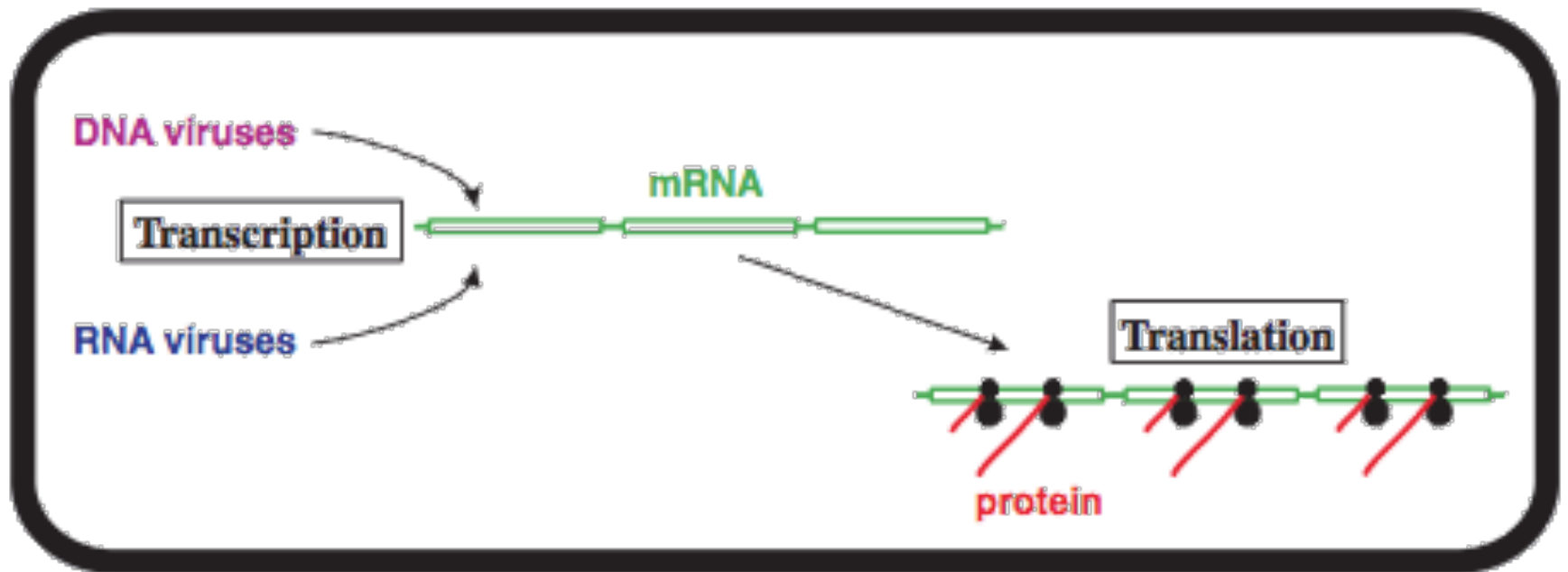
Cellular organisms



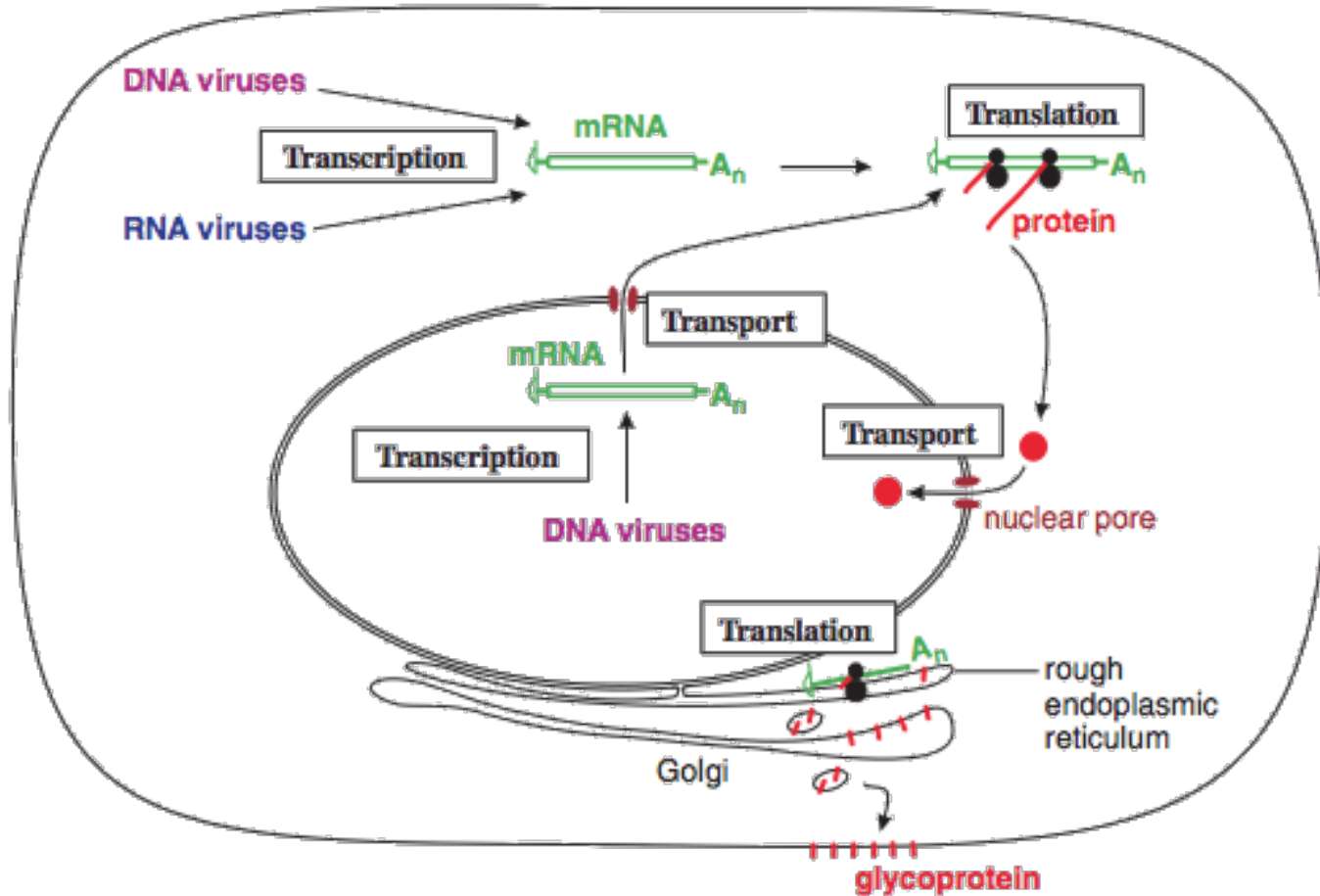
Viruses



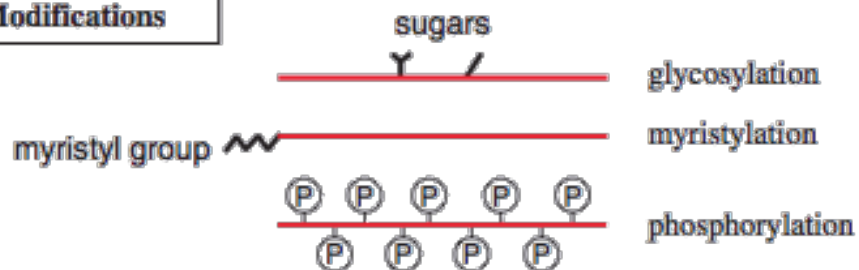
Transcription, Translation, and Transport



Transcription, Translation, and Transport

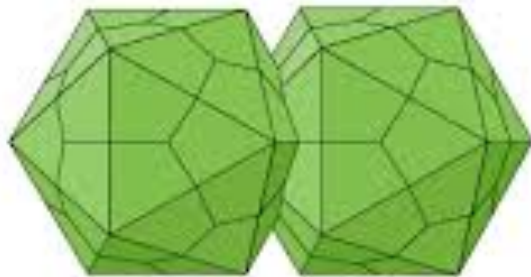


Post-Translational Modifications

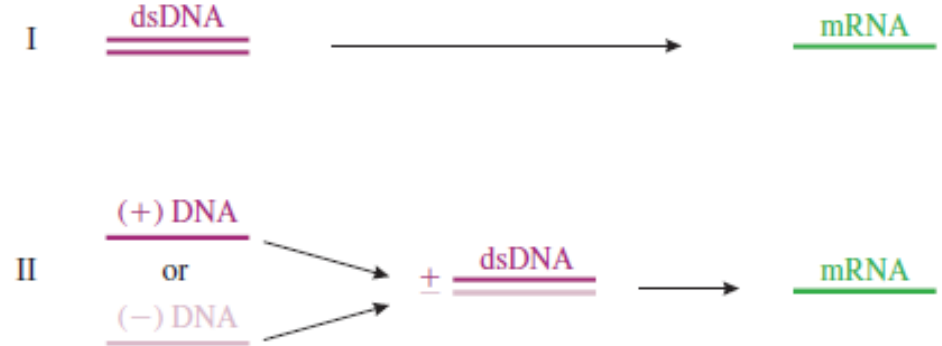


(David) Baltimore virus classification

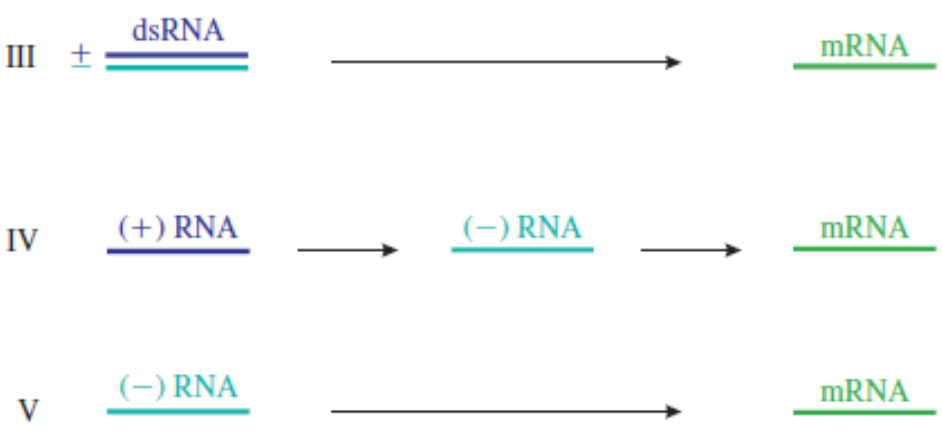
- genome type
- mode of transcription
- (-) and (+) relative to mRNA
- ambisense viruses (ssDNA - geminiviruses, arenaviruses)



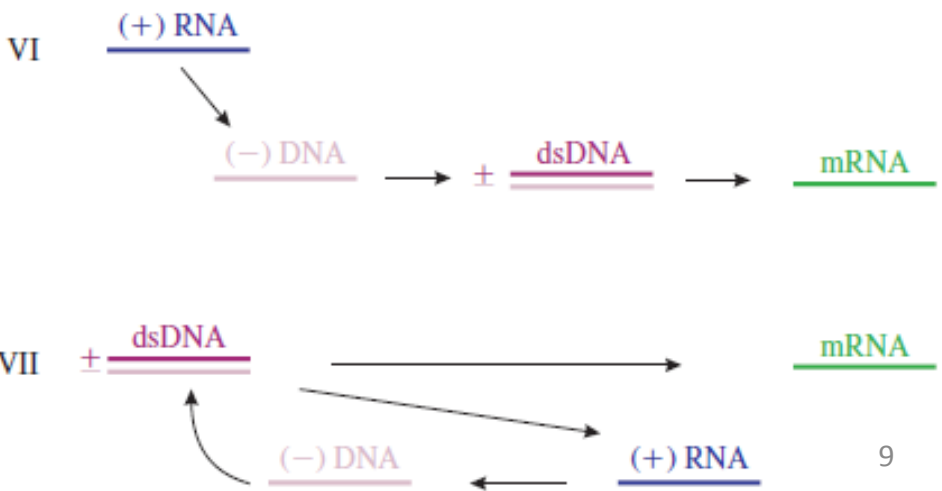
DNA Viruses



RNA Viruses

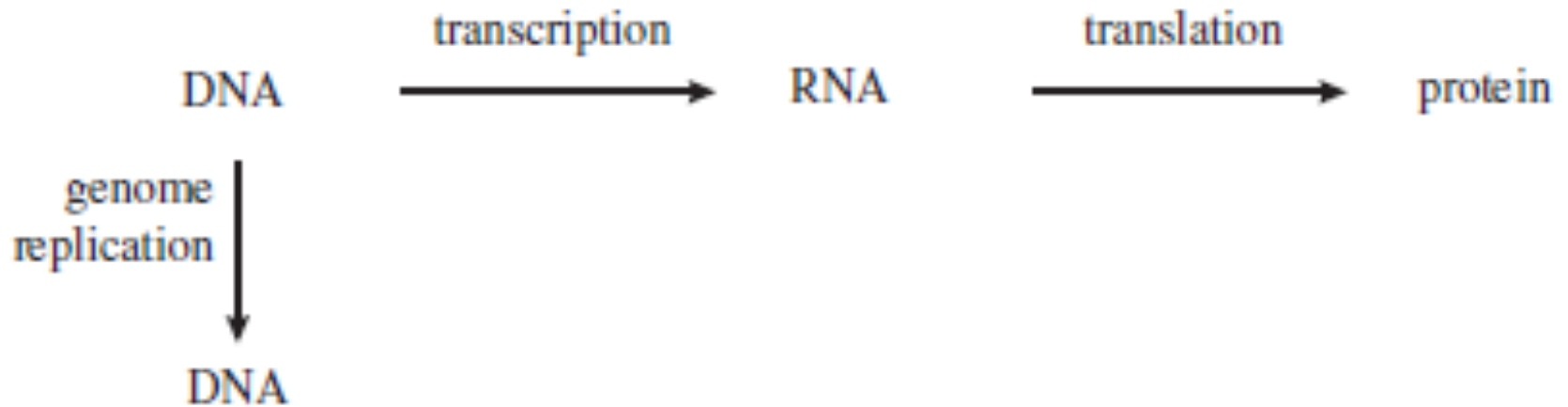


Reverse-Transcribing Viruses

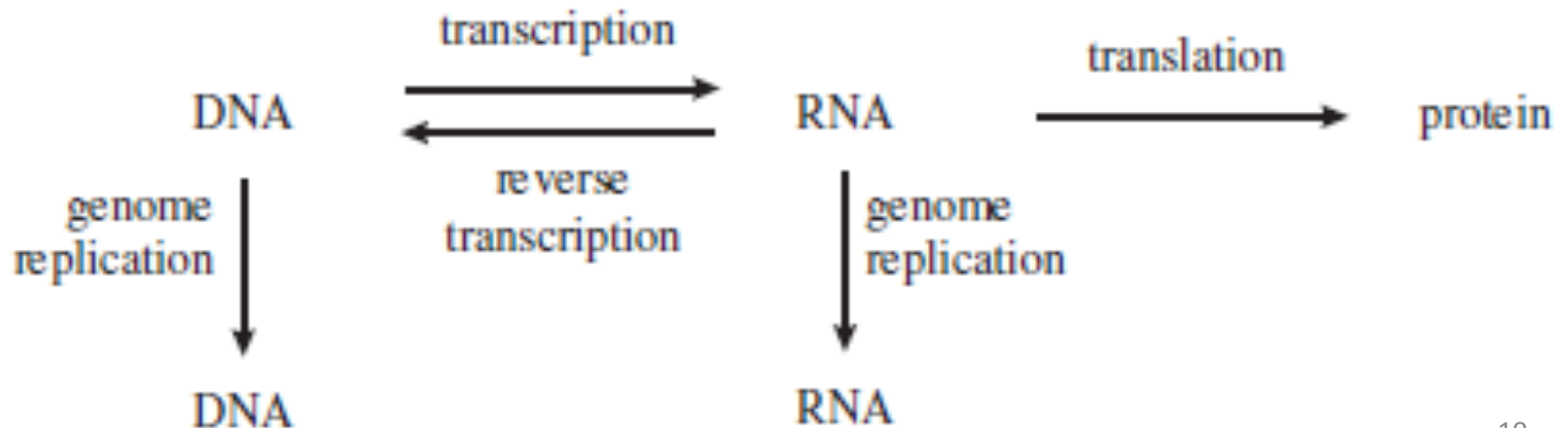


Central Dogma of Molecular Biology

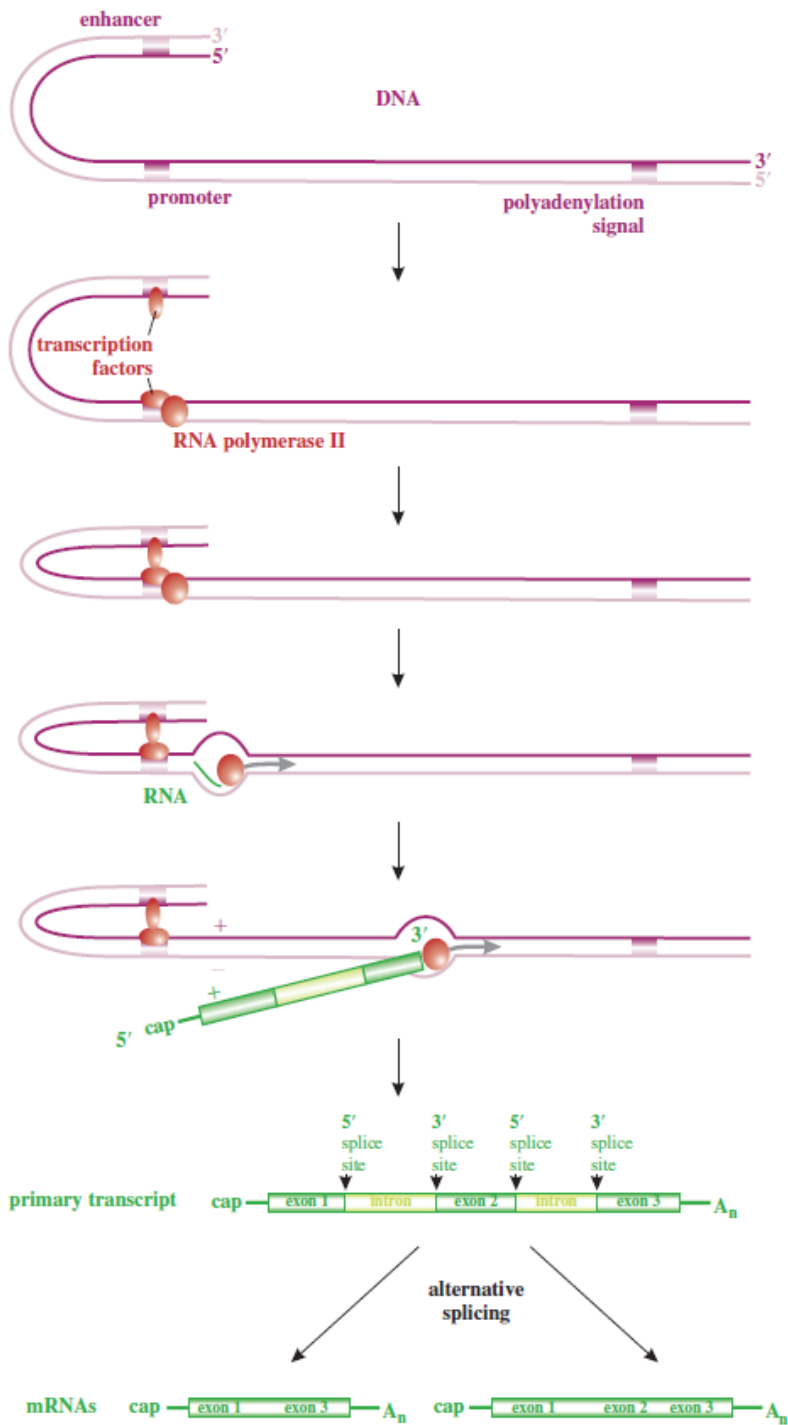
(a) Central Dogma



(b) Modified Central Dogma

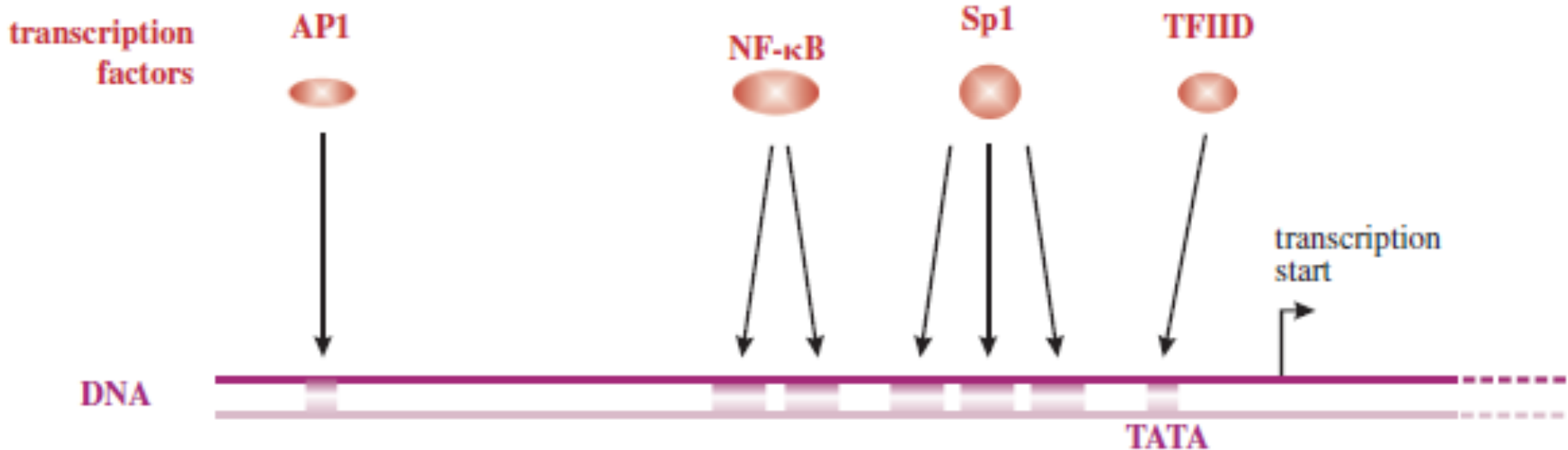


Transcription in Eukaryotes



Promoters, Enhancers, and TATA box

T A T A A/T A A/T A/G



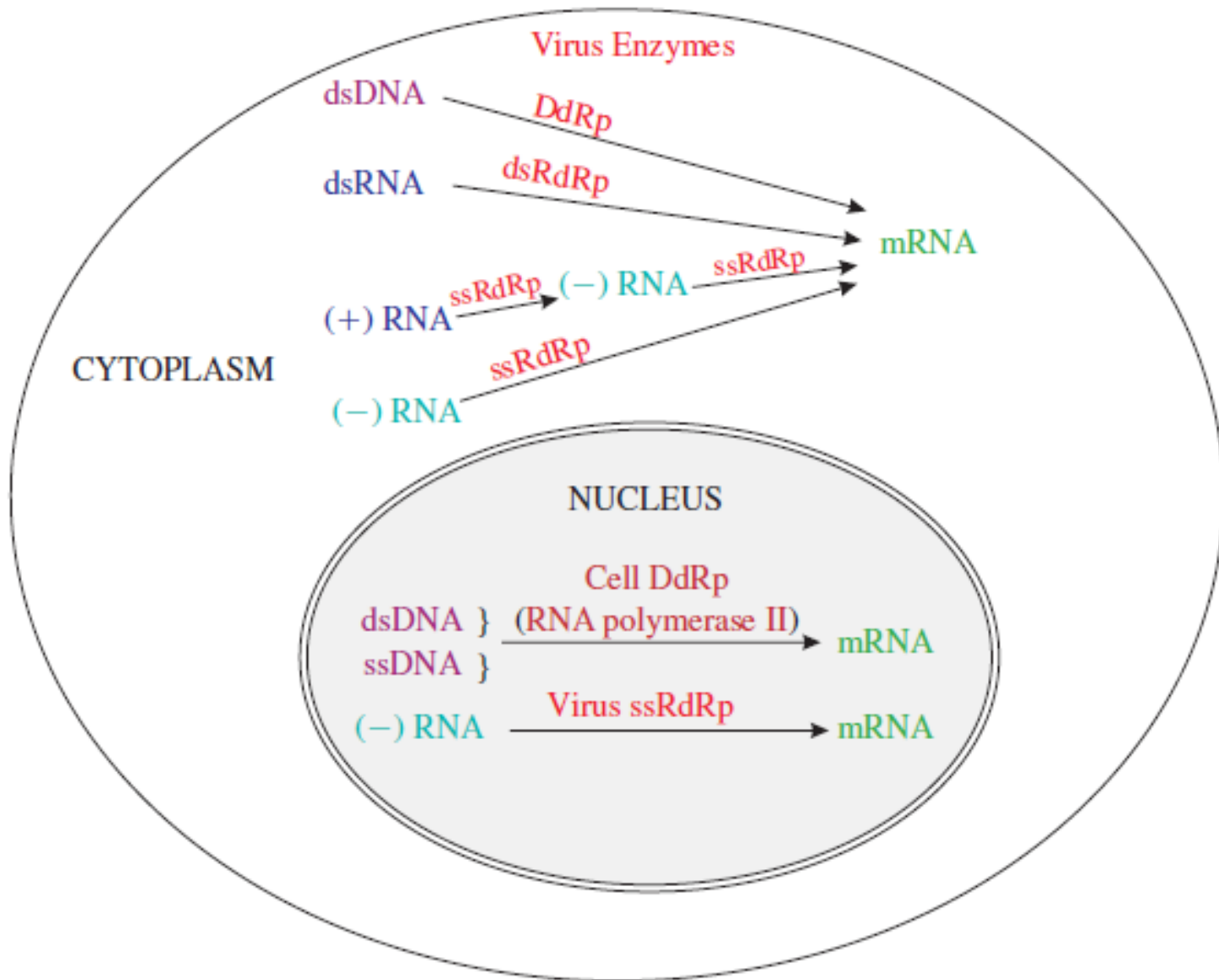
General cellular transcription factors: TFIID

Specific cellular transcription factors.

Virus transcription factors: VP16 of herpes simplex virus

- different transcription factors used during different stages of infection

Transcriptases



Obtaining mRNA cap

Cell enzymes:

- RNA triphosphatase
- Guanylyl transferase
- Methyl transferase(s)

Influenza virus – cap snatching

Poxviruses, coronaviruses, reoviruses replicate in cytoplasm and encode own capping enzymes.

Cap snatching in cytoplasm – bunyaviruses

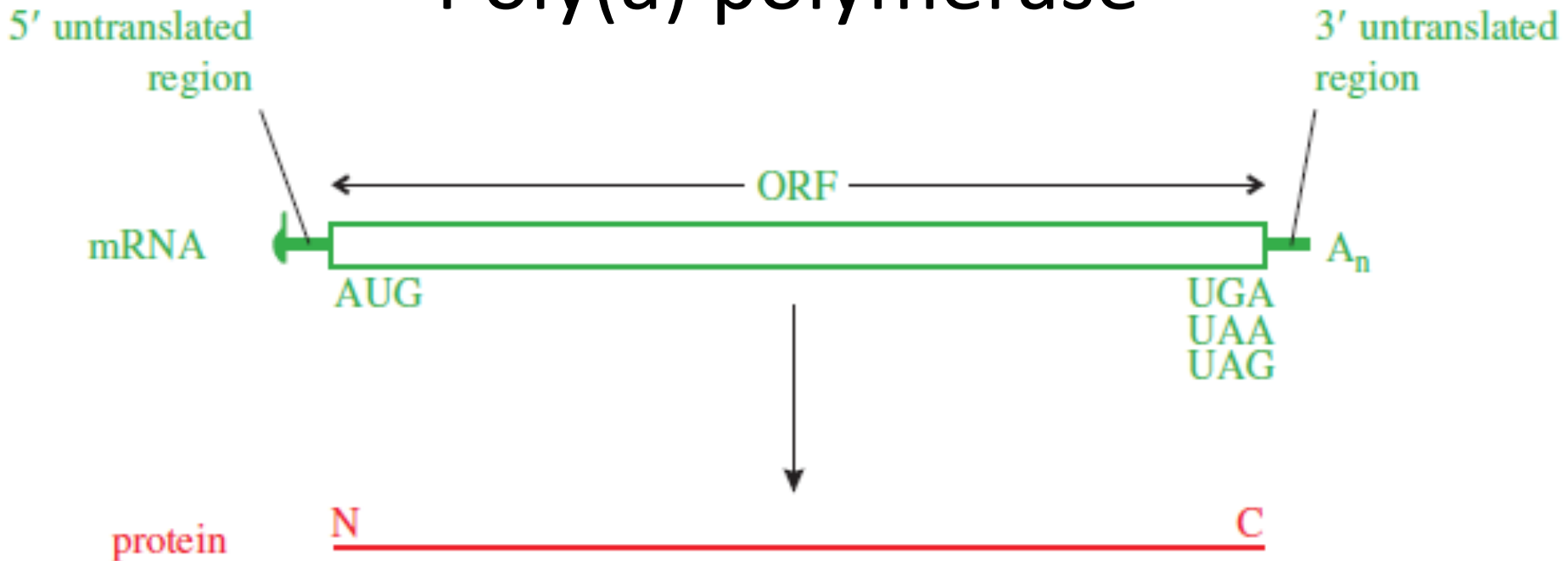
Non-capped mRNAs – picornaviruses

De-capping of host mRNAs – totiviruses

mRNA polyadenylation

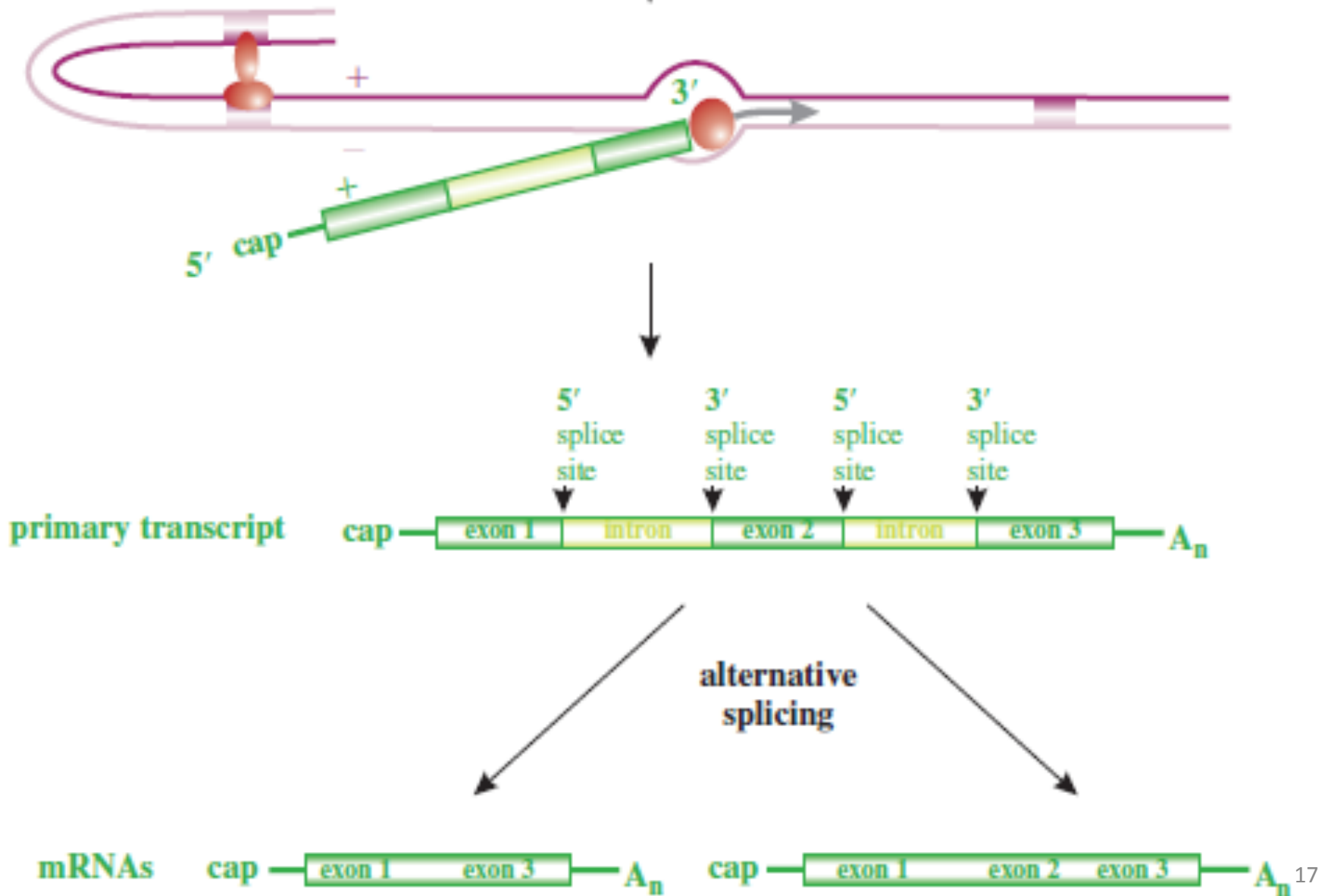
A A T A A A

Poly(a) polymerase

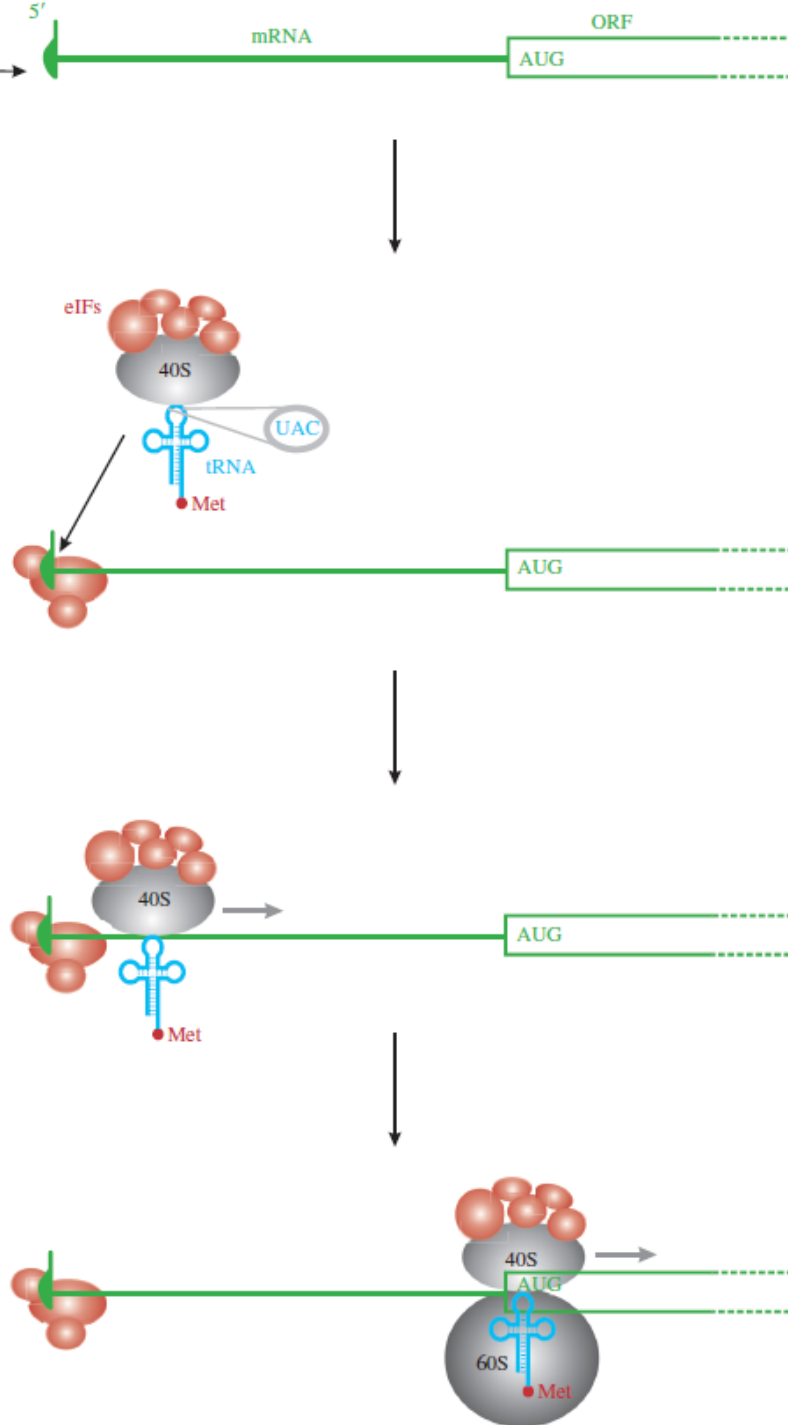


First identified in SV40 transcripts in 1981.

Pre-mRNA splicing

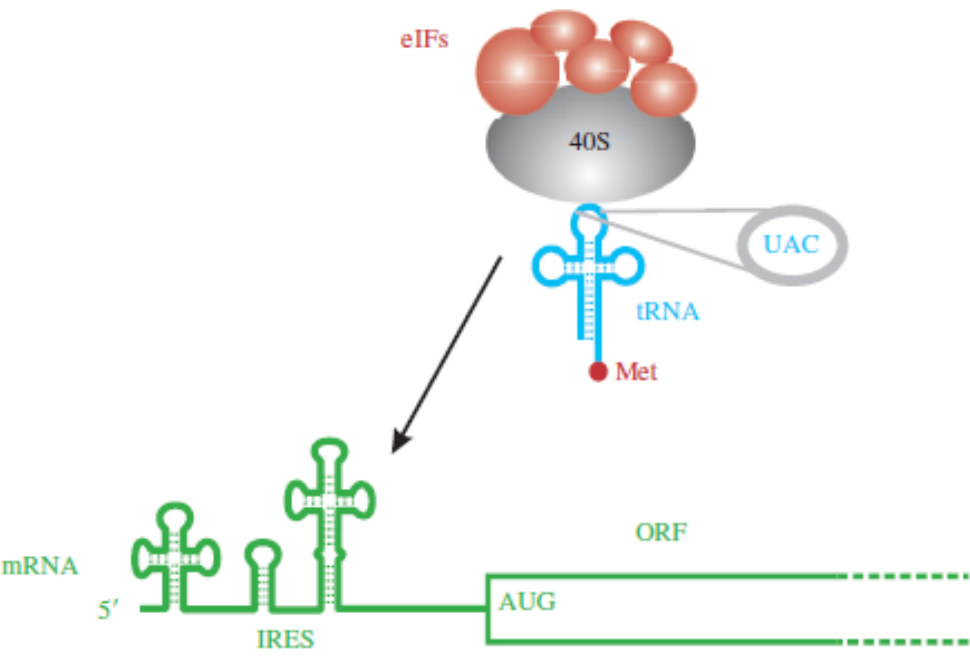


Translation

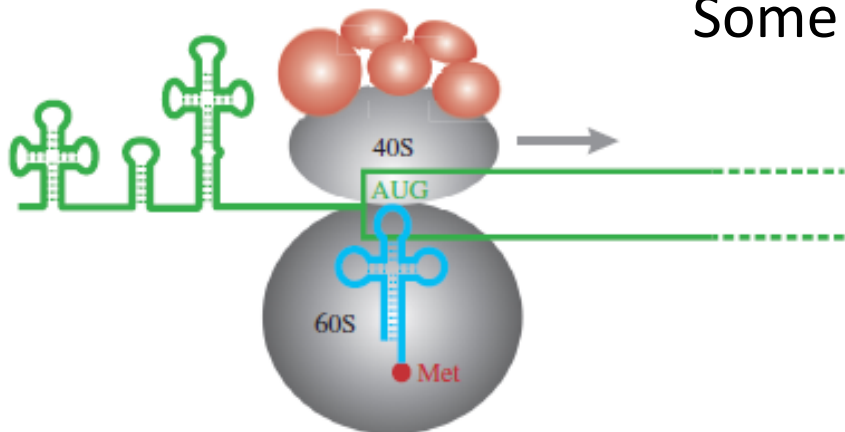


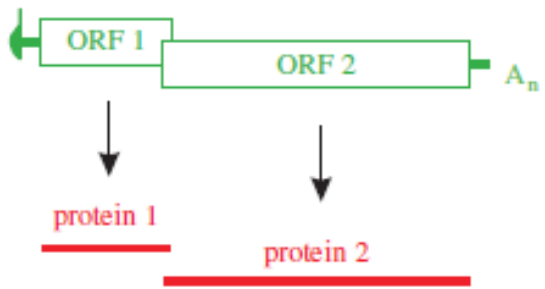
- Most viruses use host translation machinery.
- Mimiviruses encode their own tRNAs.
- eukaryotic Initiation Factors (eIF) (CAP)
- polyA binding proteins
- mRNA circularization
- 5' → 3' scanning by 40S subunit (AUG) x Sendai virus (ACG)
- IRES

IRES dependent Translation



Hepatitis C virus
Picornaviruses
Kaposi's sarcoma associated herpesvirus
Some cellular mRNAs

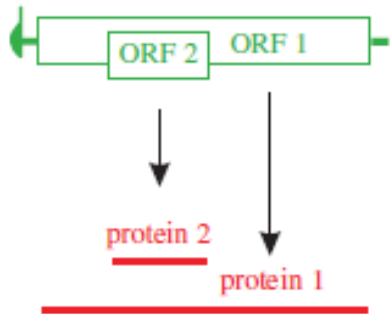




Leaky scanning

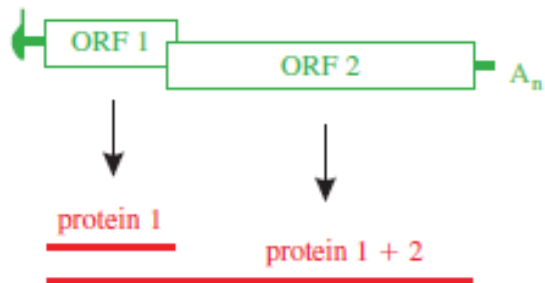
HIV-1 Vpu and Env
(Chapter 18)

Bicistronic mRNAs



Leaky scanning

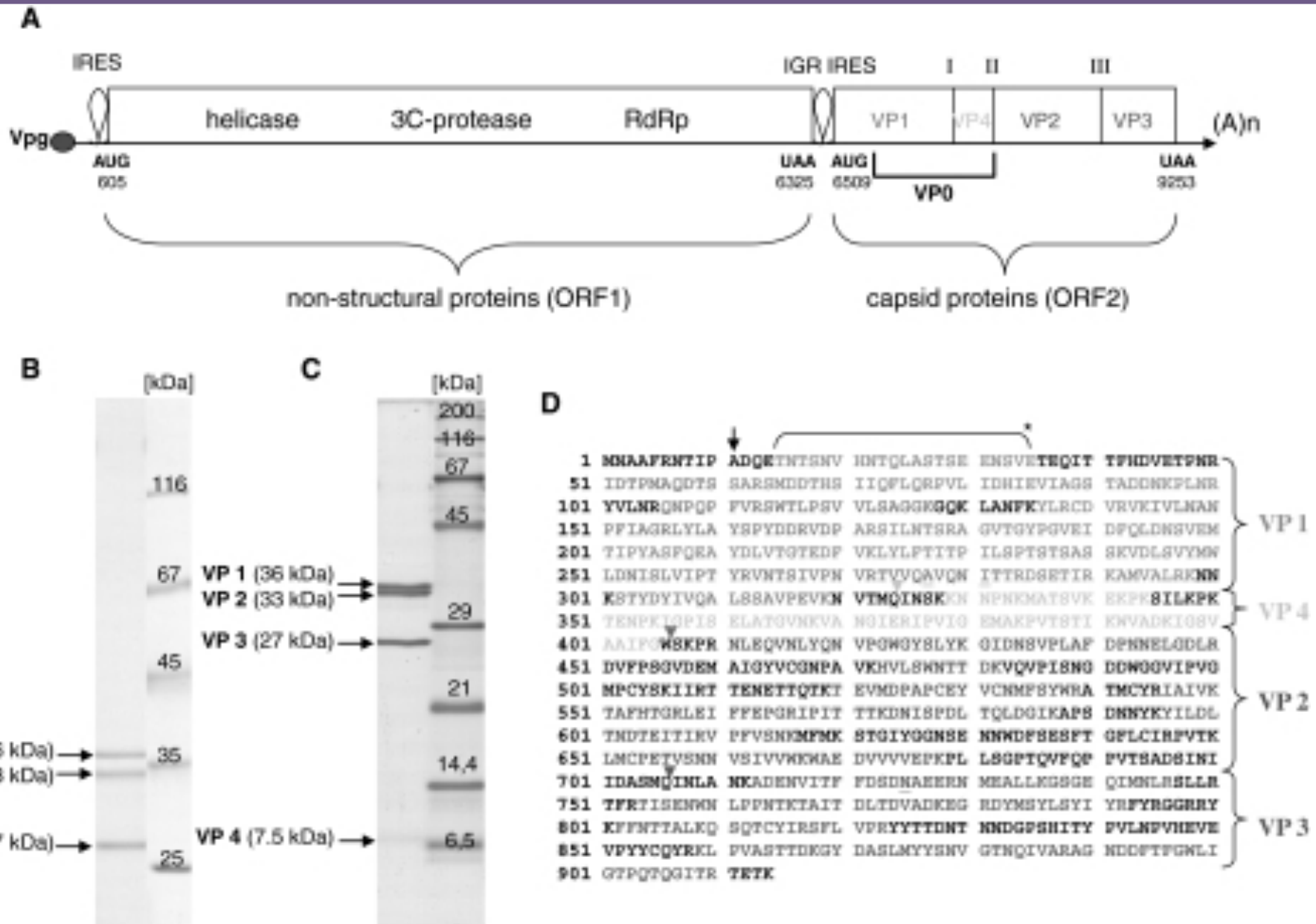
Rotavirus NSP5 and NSP6
(Chapter 13)



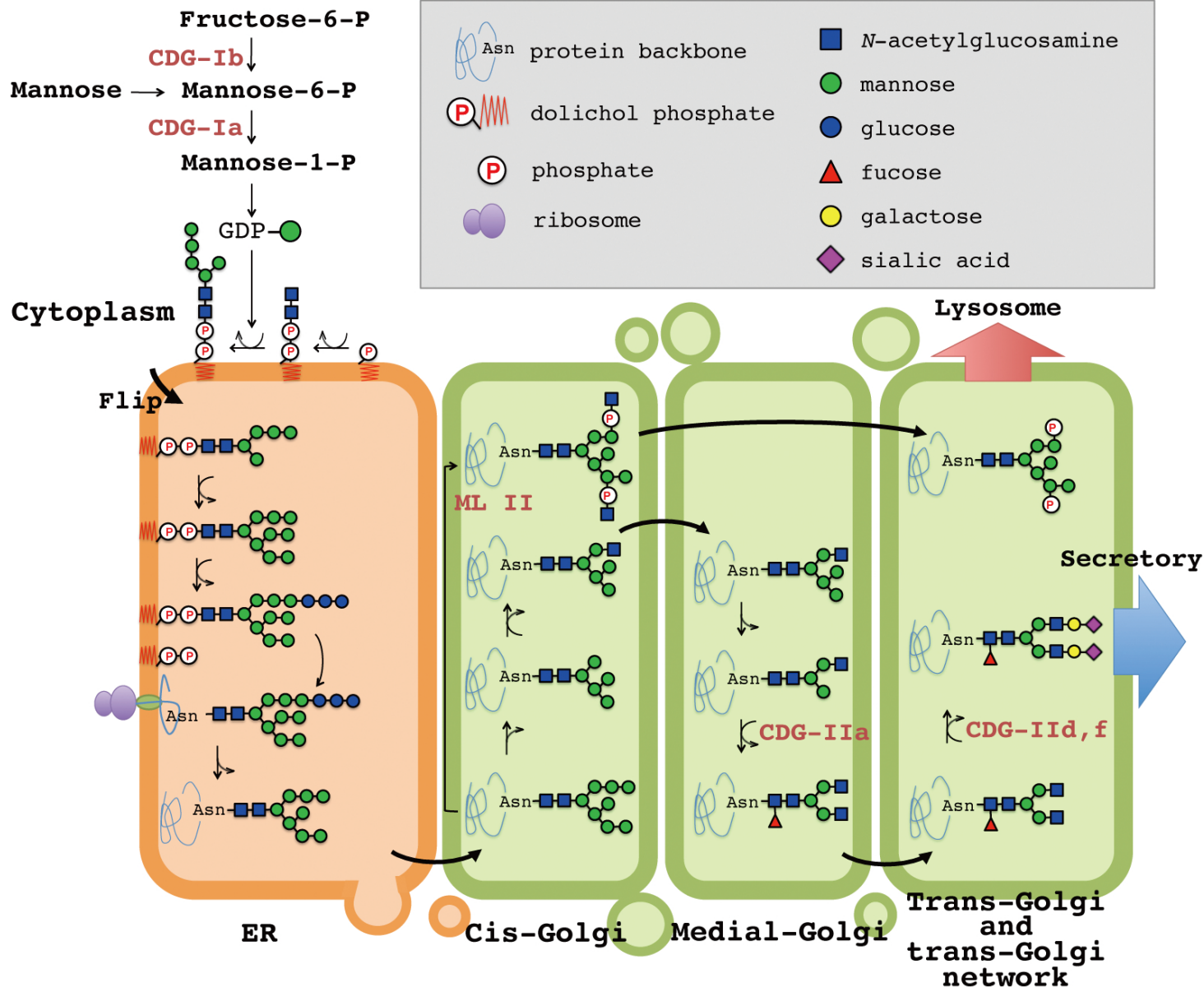
Ribosomal frameshift

HIV-1 Gag and Gag-Pol
(Chapter 18)

Bicistronic mRNAs of dicistroviruses

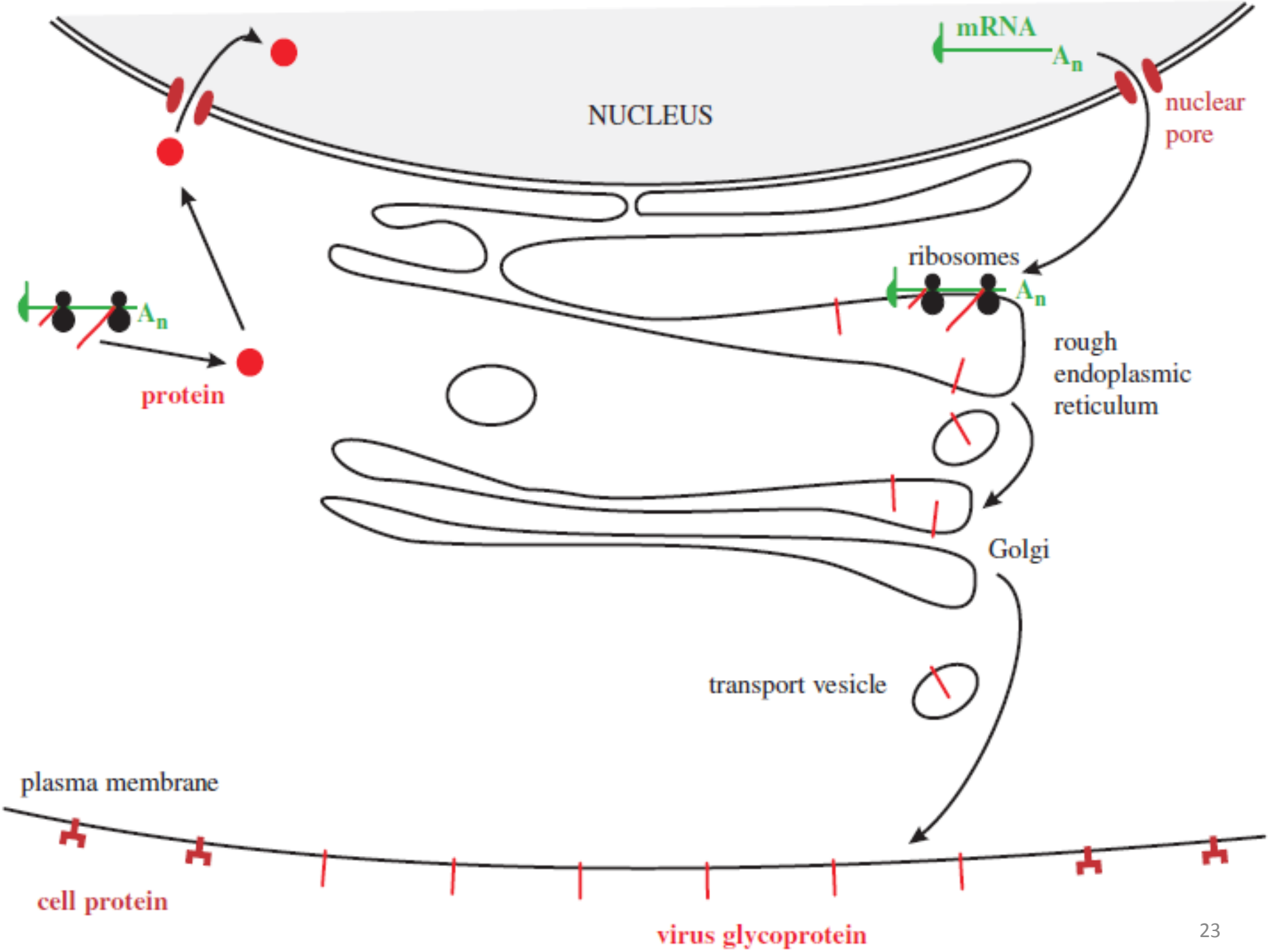


Co- and post translational protein modification



N-glycosylation
(Asn)

O-glycosylation
(Tyr, Ser)



Co- and post translational protein modification

Acylation (addition of Myristic acid)

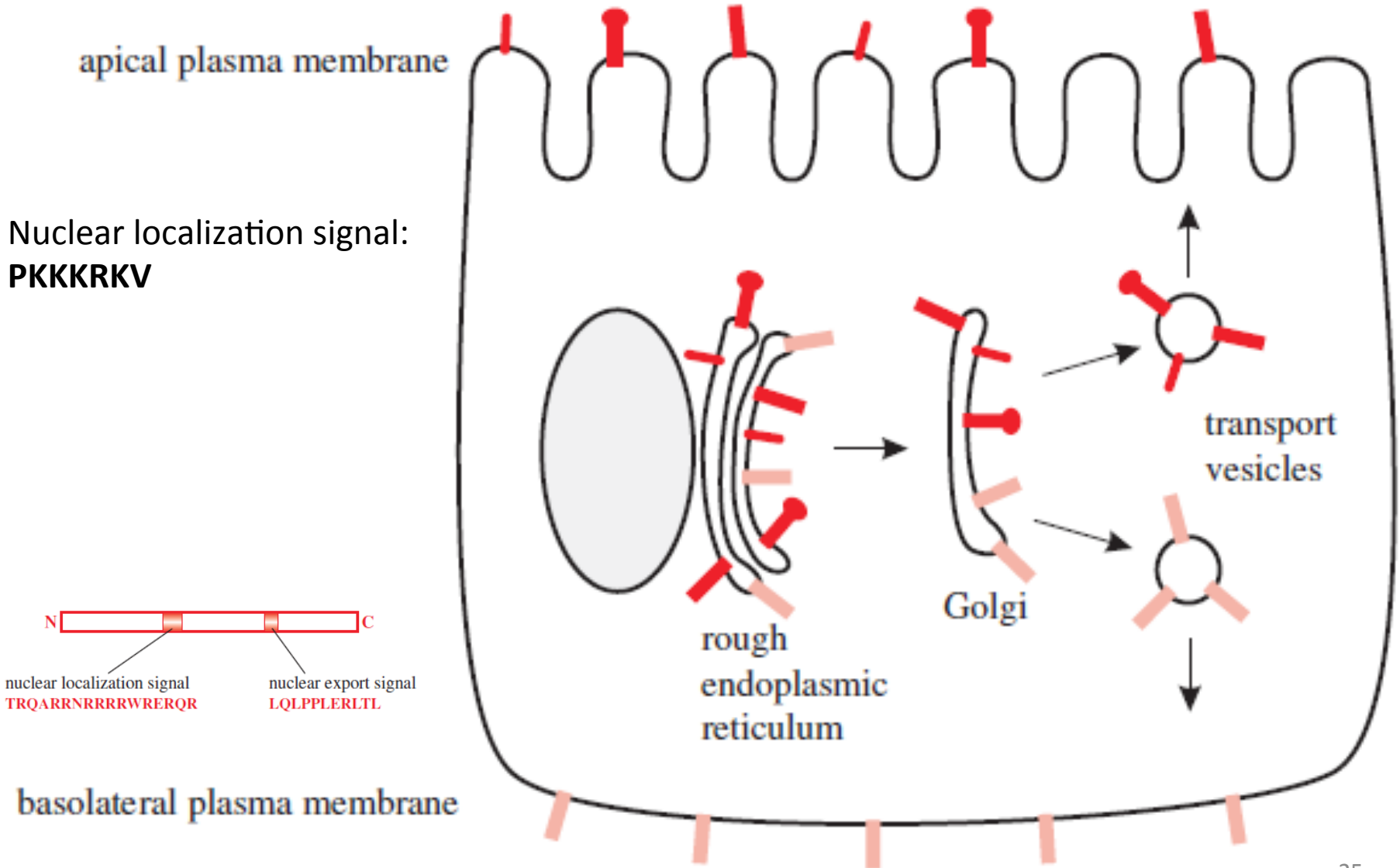
- Gag of HIV
- VP4 of picornaviruses

Phosphorylation

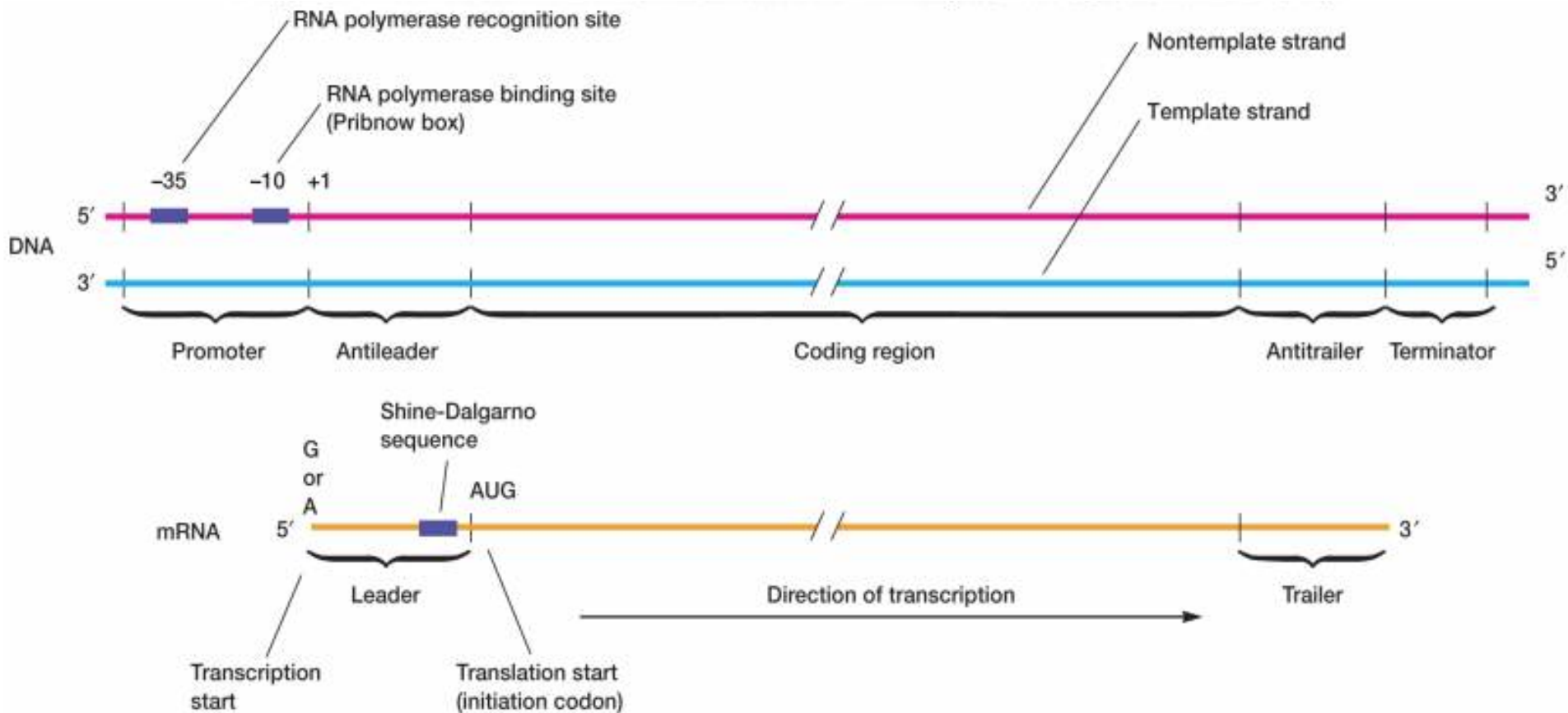
- serine, threonine, tyrosine

Cleavage

Targeting of virus proteins



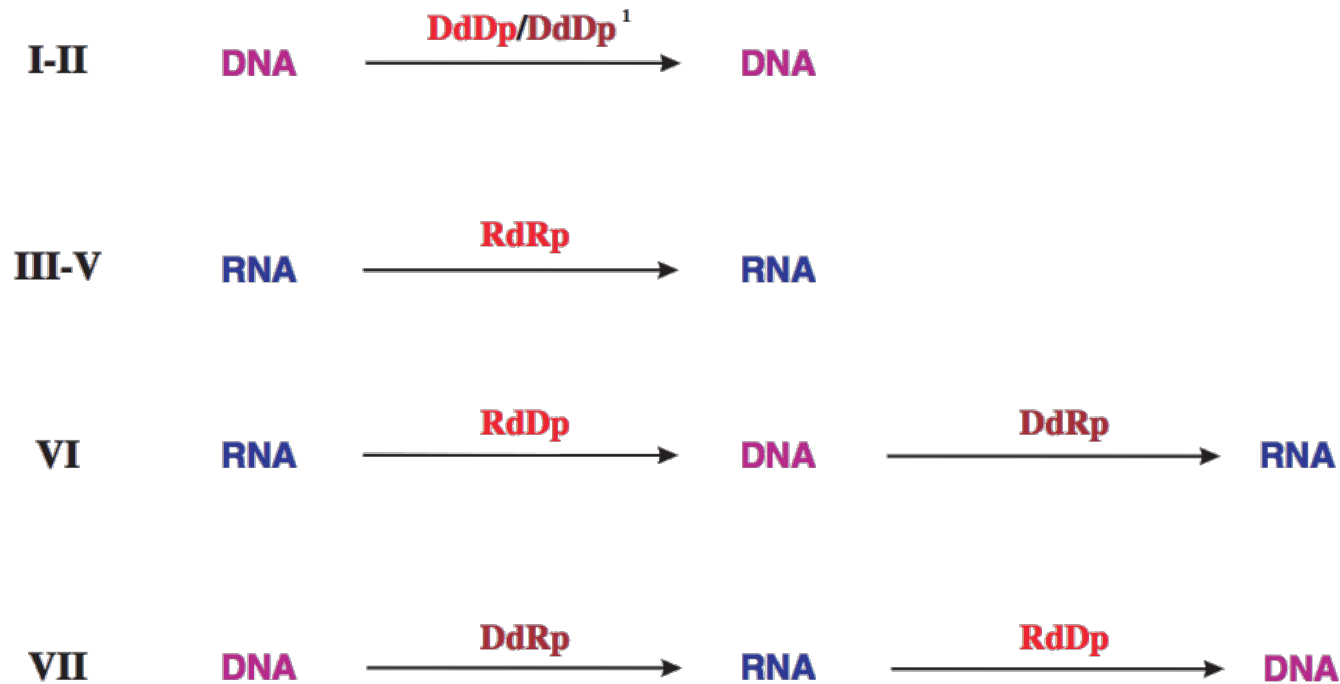
Transcription in bacteria



Learning outcomes

- explain how virus genes are transcribed and translated
- describe the post-translational modifications that some virus proteins undergo
- highlight differences in transcription and translation between prokaryotic and eukaryotic cells
- discuss the transport of virus proteins and RNA within cells

Virus Genome Replication



Virus enzymes: DdDp = DNA-dependent DNA polymerase
RdRp = RNA-dependent RNA polymerase
RdDp = RNA-dependent DNA polymerase (reverse transcriptase)

Cell enzymes: DdDp = DNA-dependent DNA polymerase
DdRp = DNA-dependent RNA polymerase (RNA pol II)

¹ Some dsDNA viruses use a cell DdDp, some encode their own.

CYTOPLASM

some dsDNA viruses

dsRNA viruses

(+) RNA viruses

(-) RNA viruses (non-segmented genomes)

retroviruses & pararetroviruses (RNA → DNA)

NUCLEUS

NUCLEUS

some dsDNA viruses

ssDNA viruses

(-) RNA viruses (segmented genomes)

retroviruses & pararetroviruses (DNA → RNA)

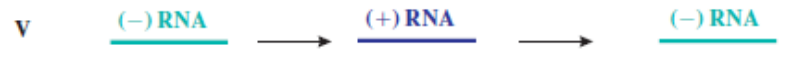
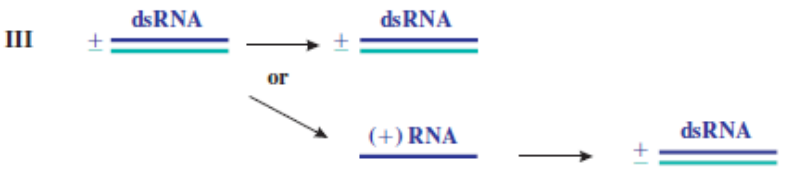
Primers



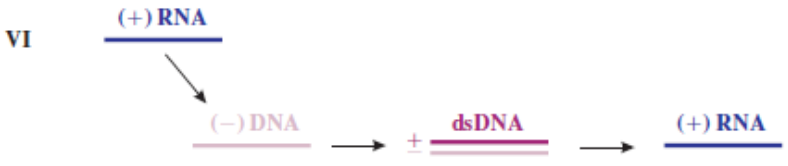
DNA Viruses



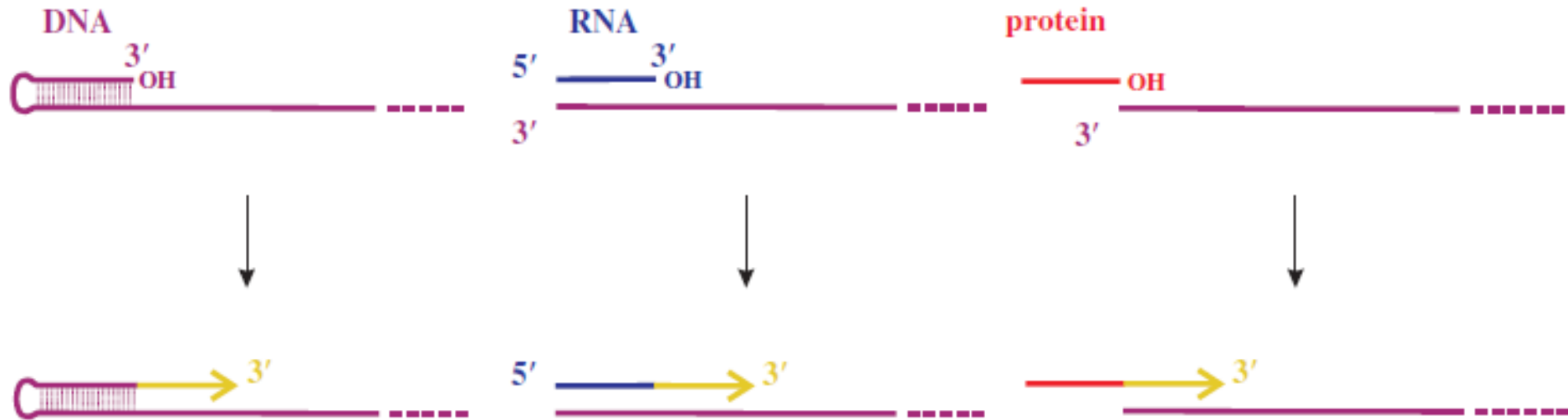
RNA Viruses



Reverse-Transcribing Viruses



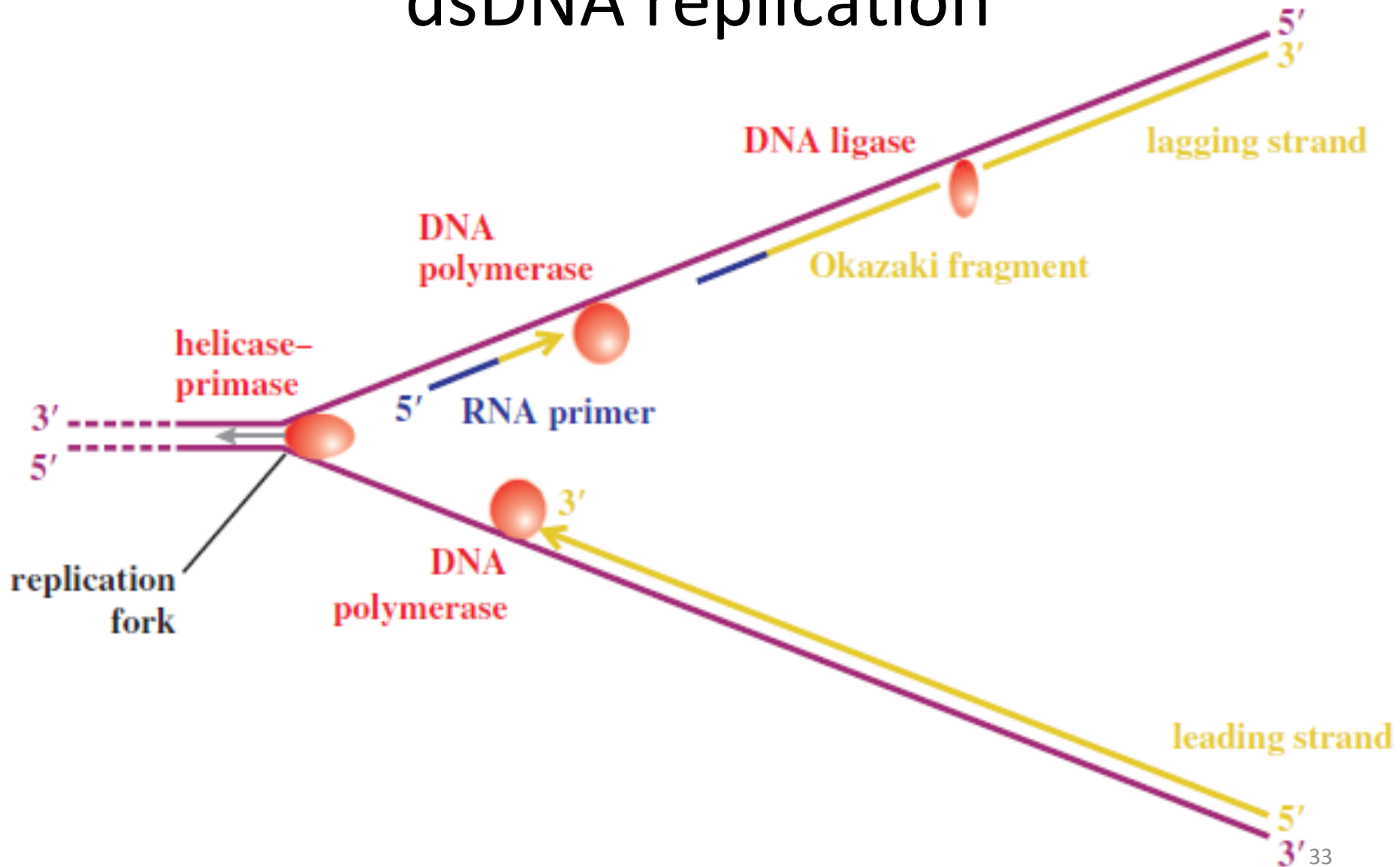
Priming of NA synthesis



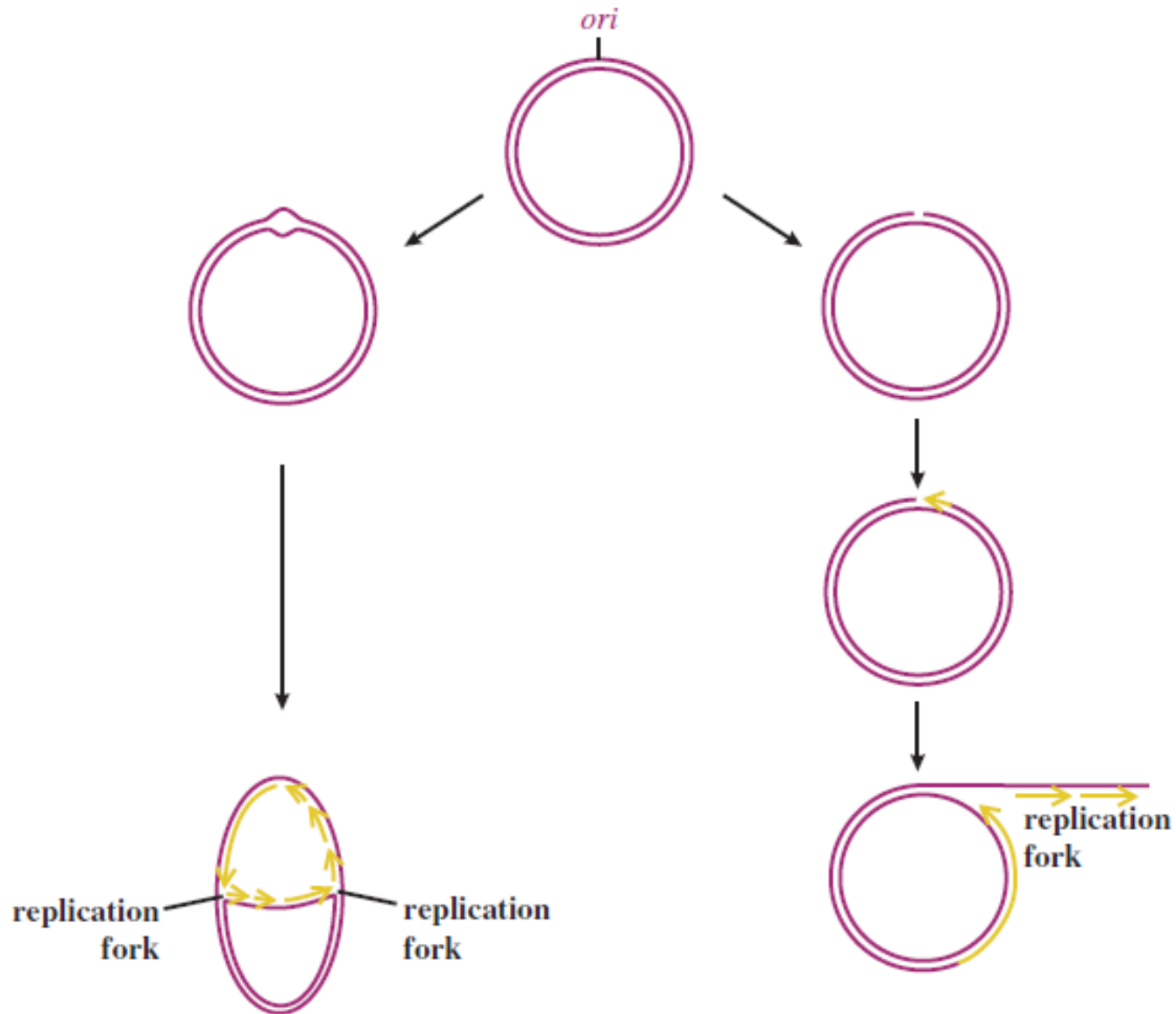
Location of virus replication sites in eucaryotes

Virus genome	Cytoplasm	Nucleus
dsDNA	Some	Some
ssDNA		All
dsRNA	All	
(+) RNA	All	
(-) RNA (non-segmented genome)	All	
(-) RNA (segmented genome)		All
Retroviruses [(+) RNA] } Pararetroviruses [dsDNA] }	ssRNA → dsDNA	dsDNA → ssRNA

Leading and Lagging strands in dsDNA replication



Rolling circle x “normal” replication



Conservative x semiconservative replication



Replication of retroviruses



Learning outcomes

- state the locations within eukaryotic cells where different categories of virus genome are replicated
- explain the role of primers in virus nucleic acid synthesis
- discuss the roles of virus and host proteins in virus genome replication
- outline the replication mechanisms of virus DNAs and RNAs
- explain the term 'reverse transcription'