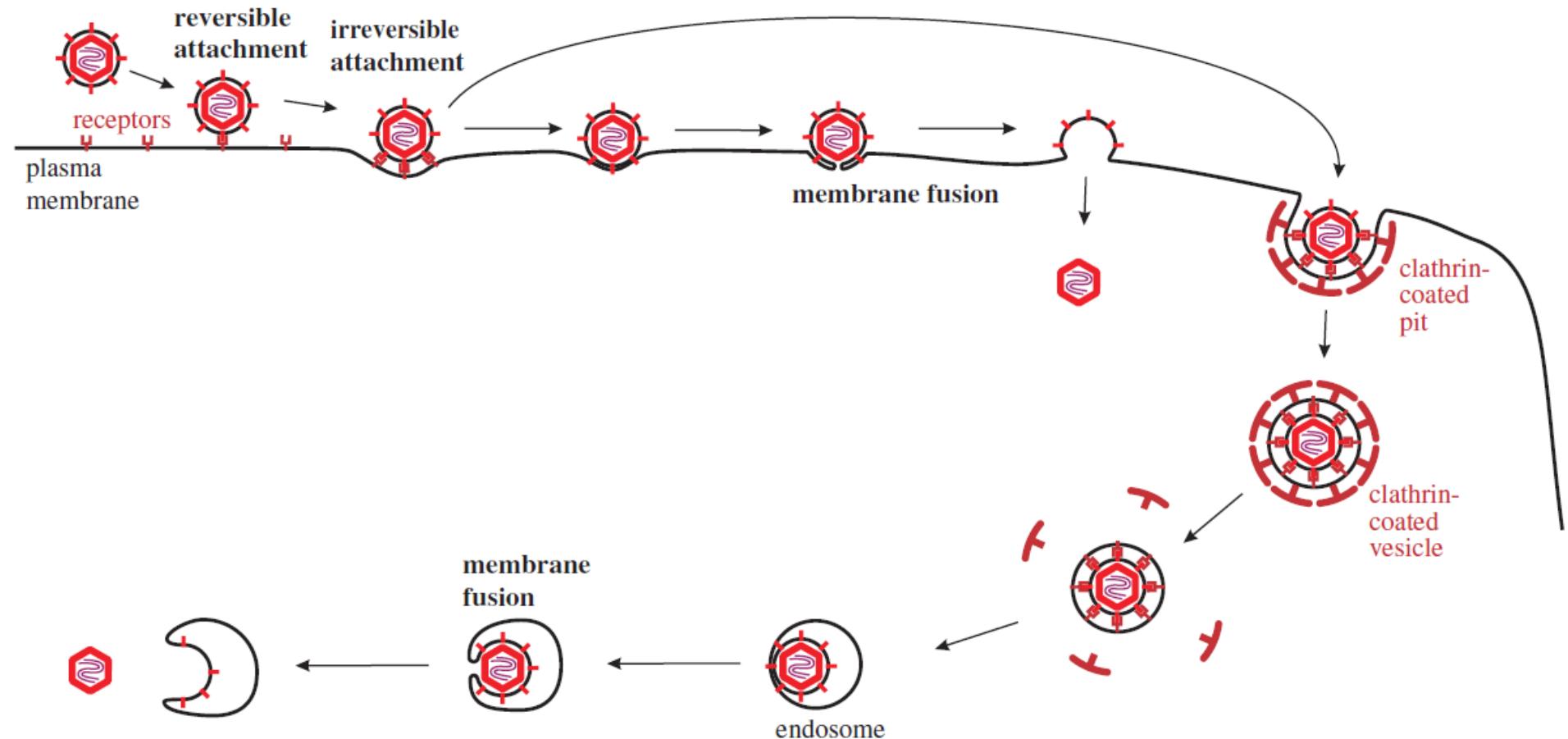


Structural Virology

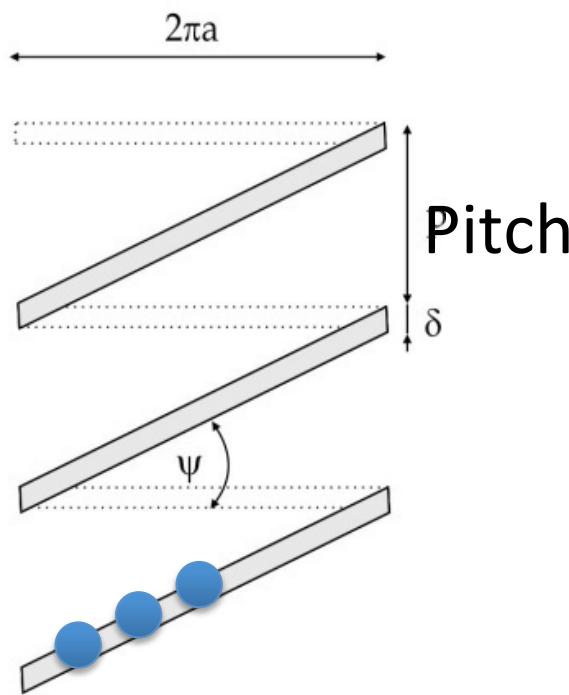
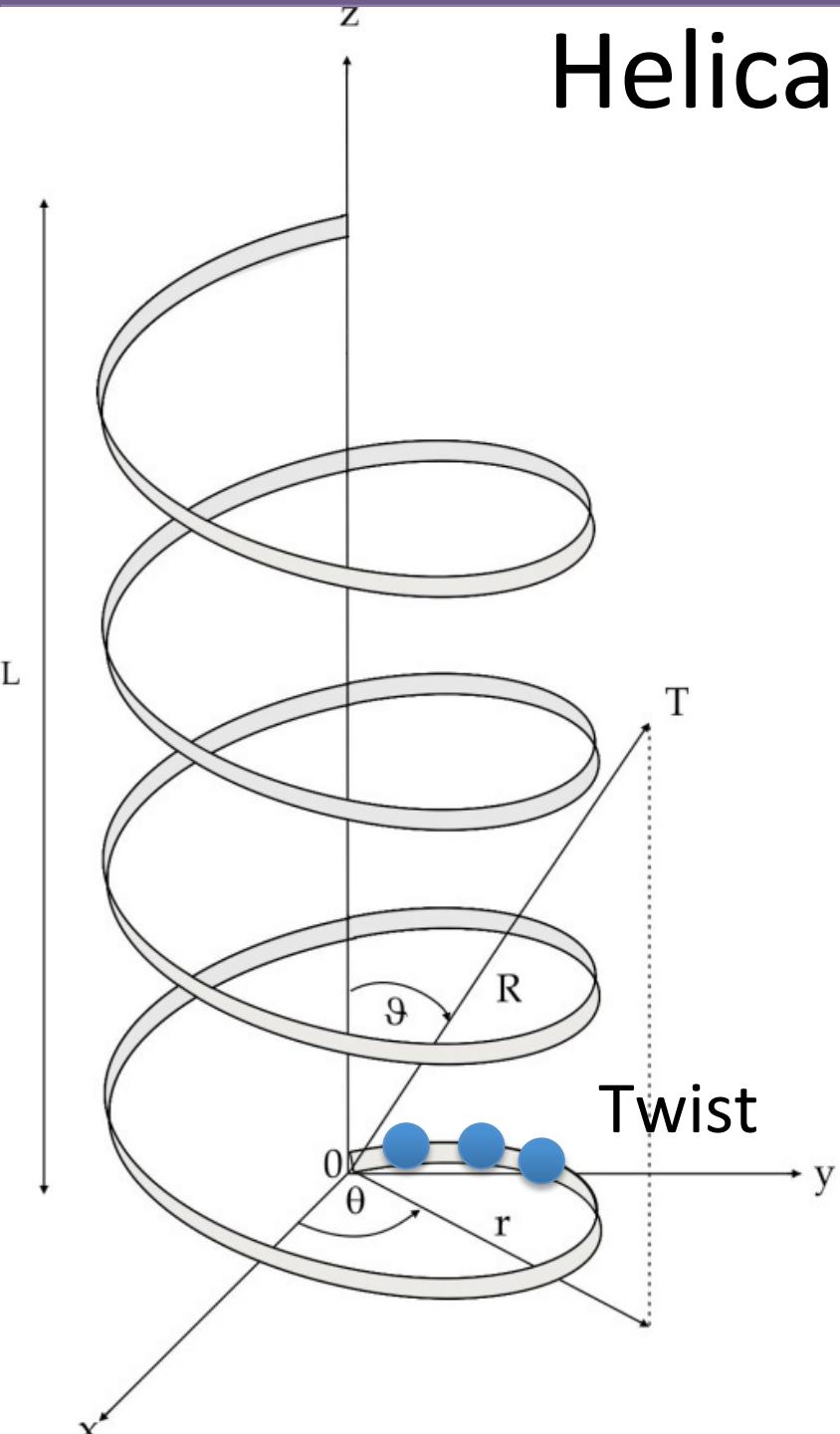
Lecture 3

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

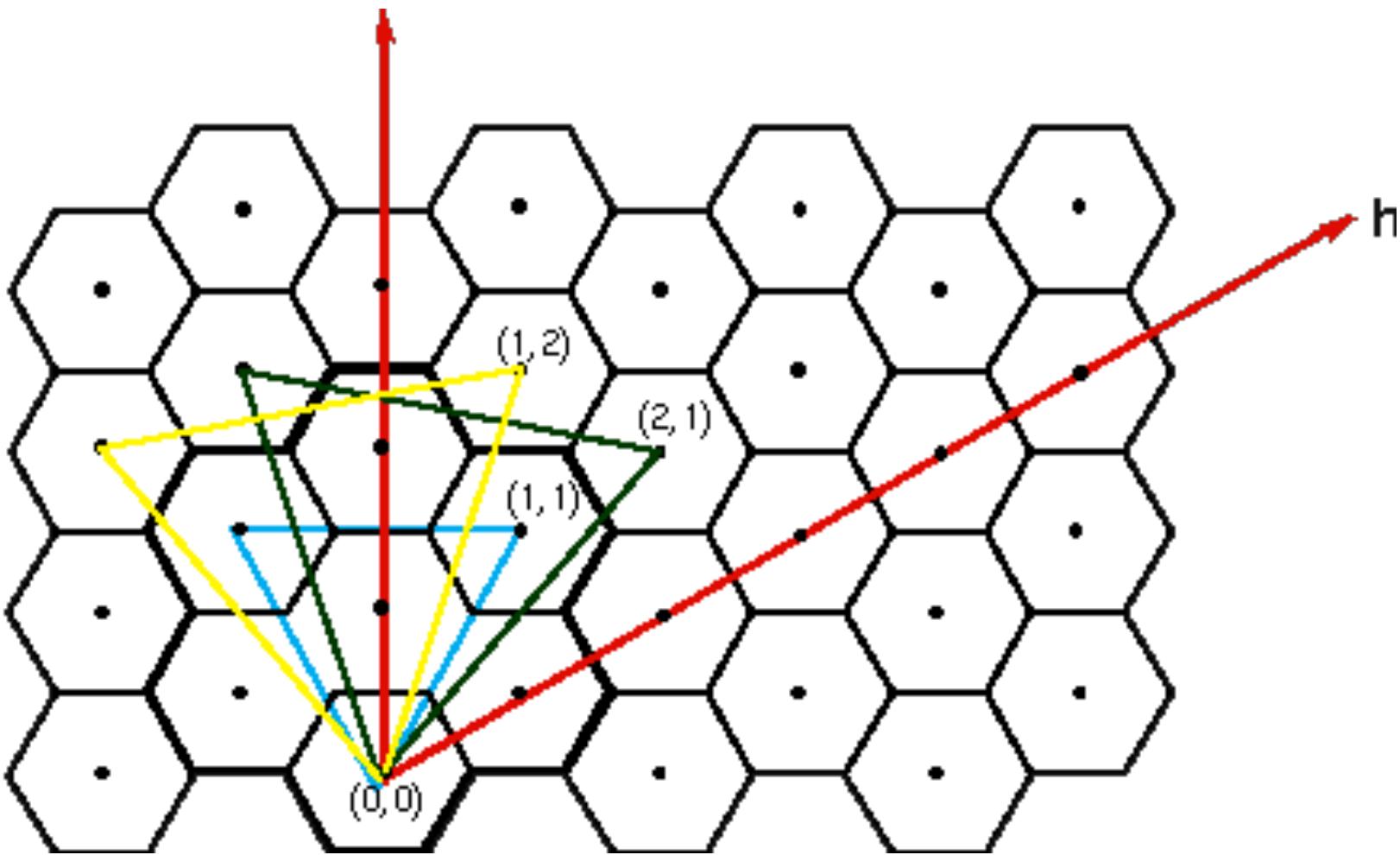


HRV16 from stock 28.11.13,
PFU $5 \cdot 10^3$

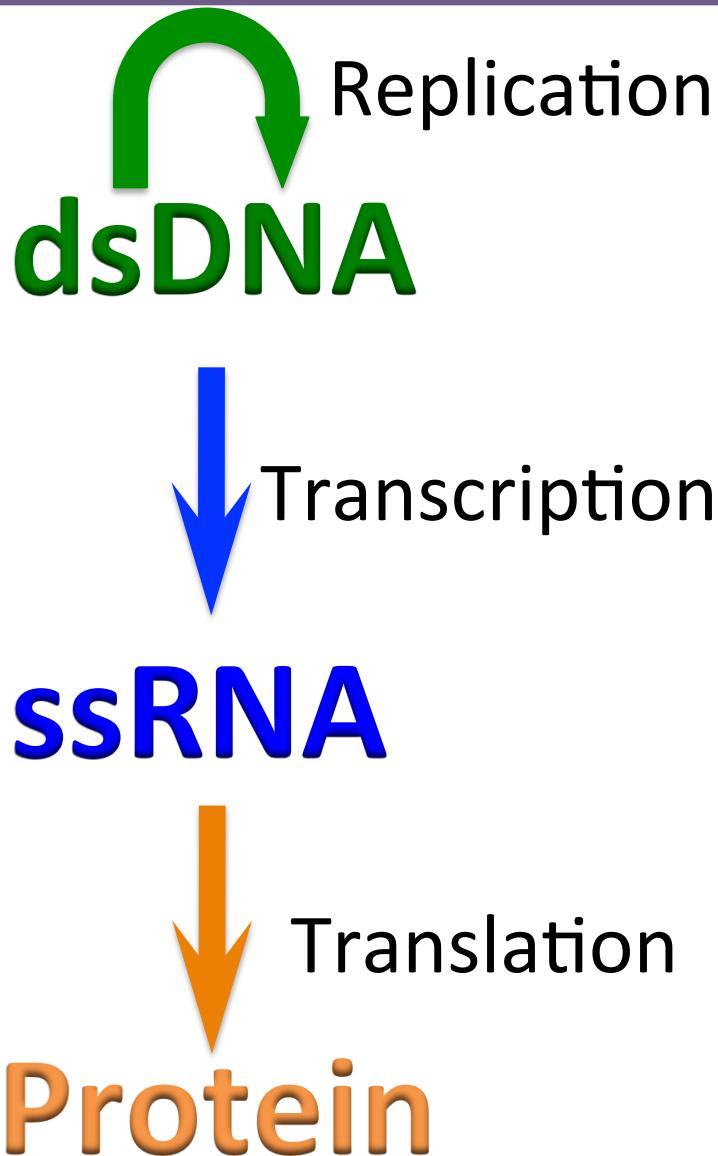
Quasi equivalence

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

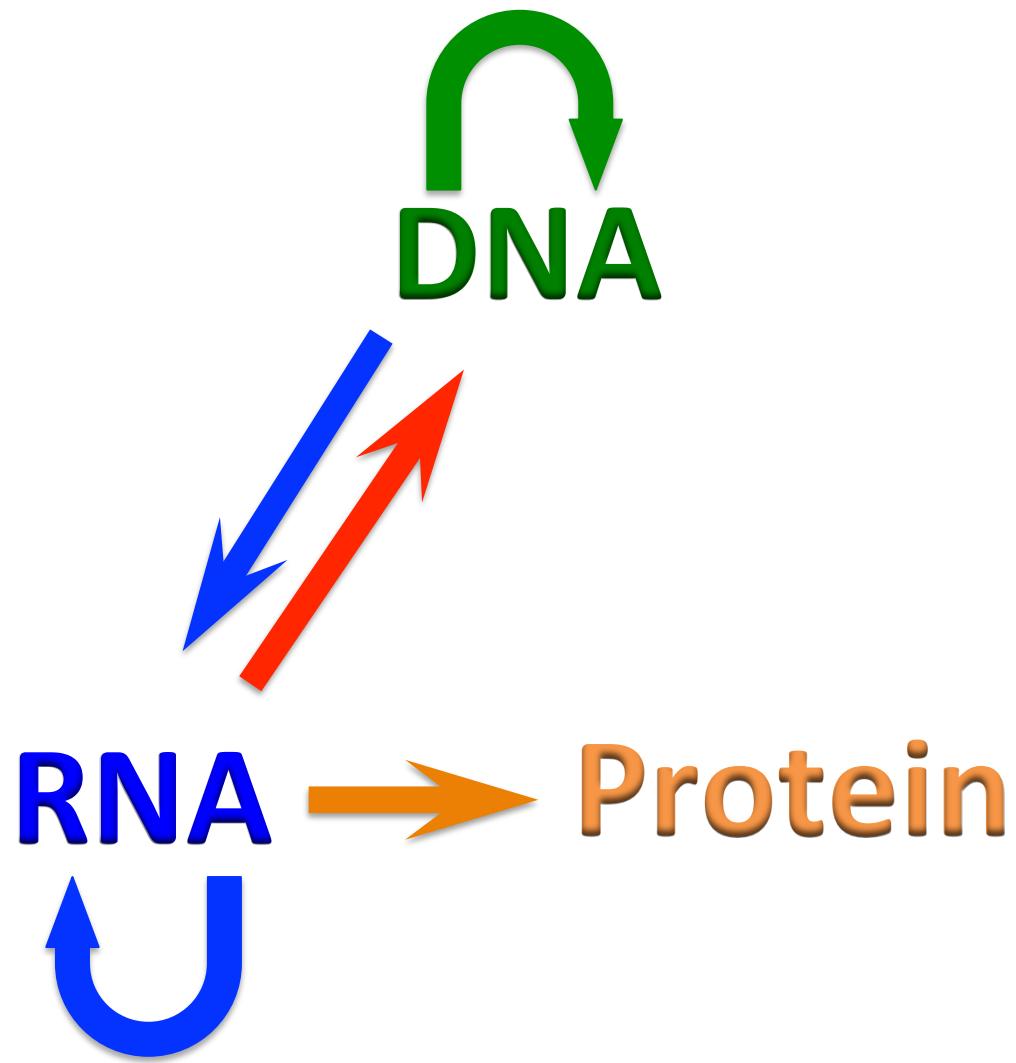
a)



Cellular organisms



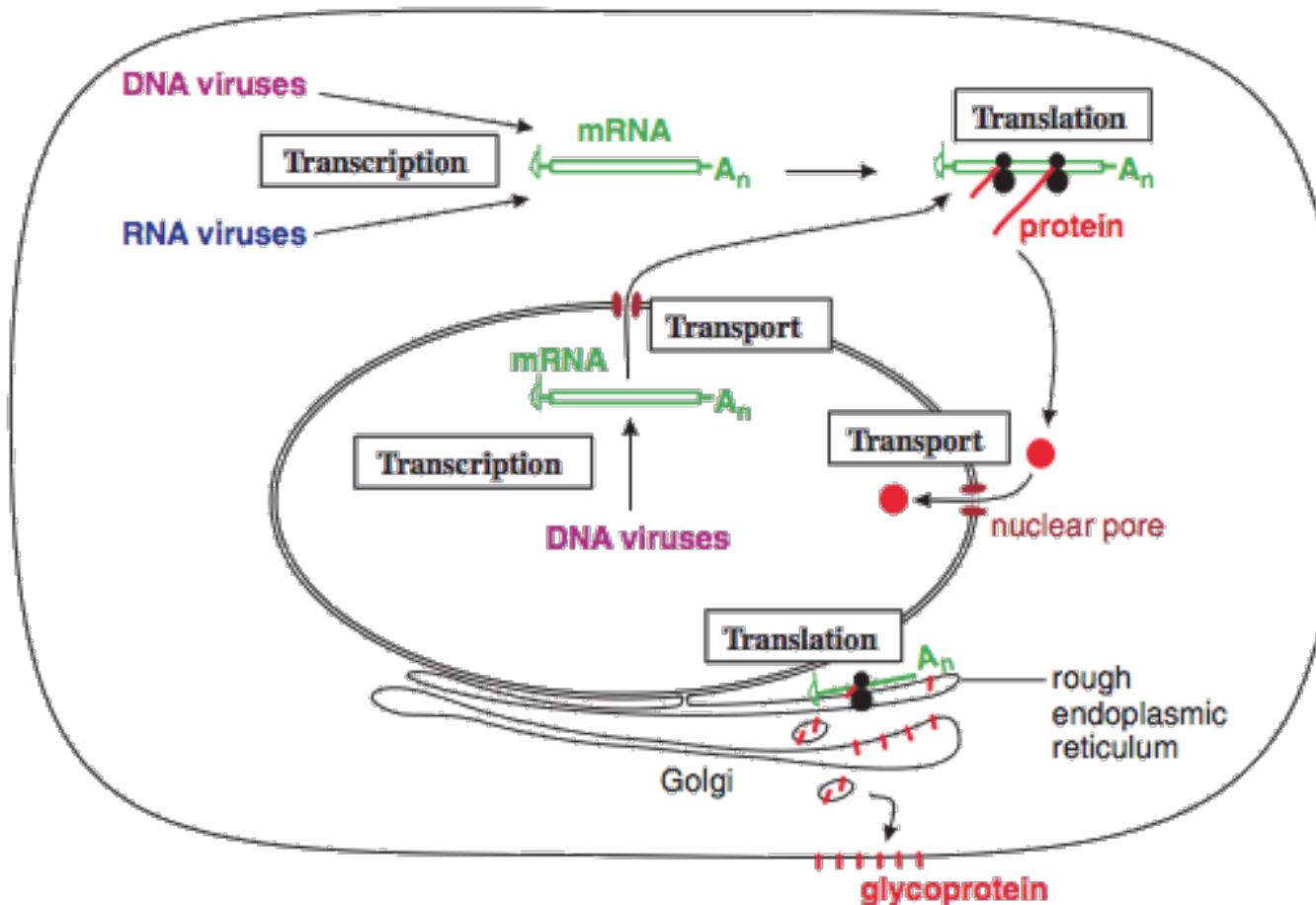
Viruses



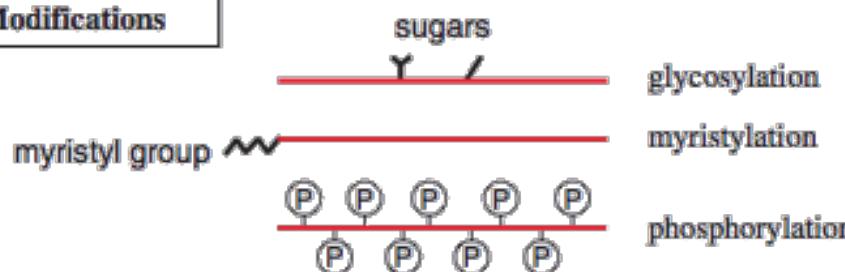
Transcription, Translation, and Transport



Transcription, Translation, and Transport

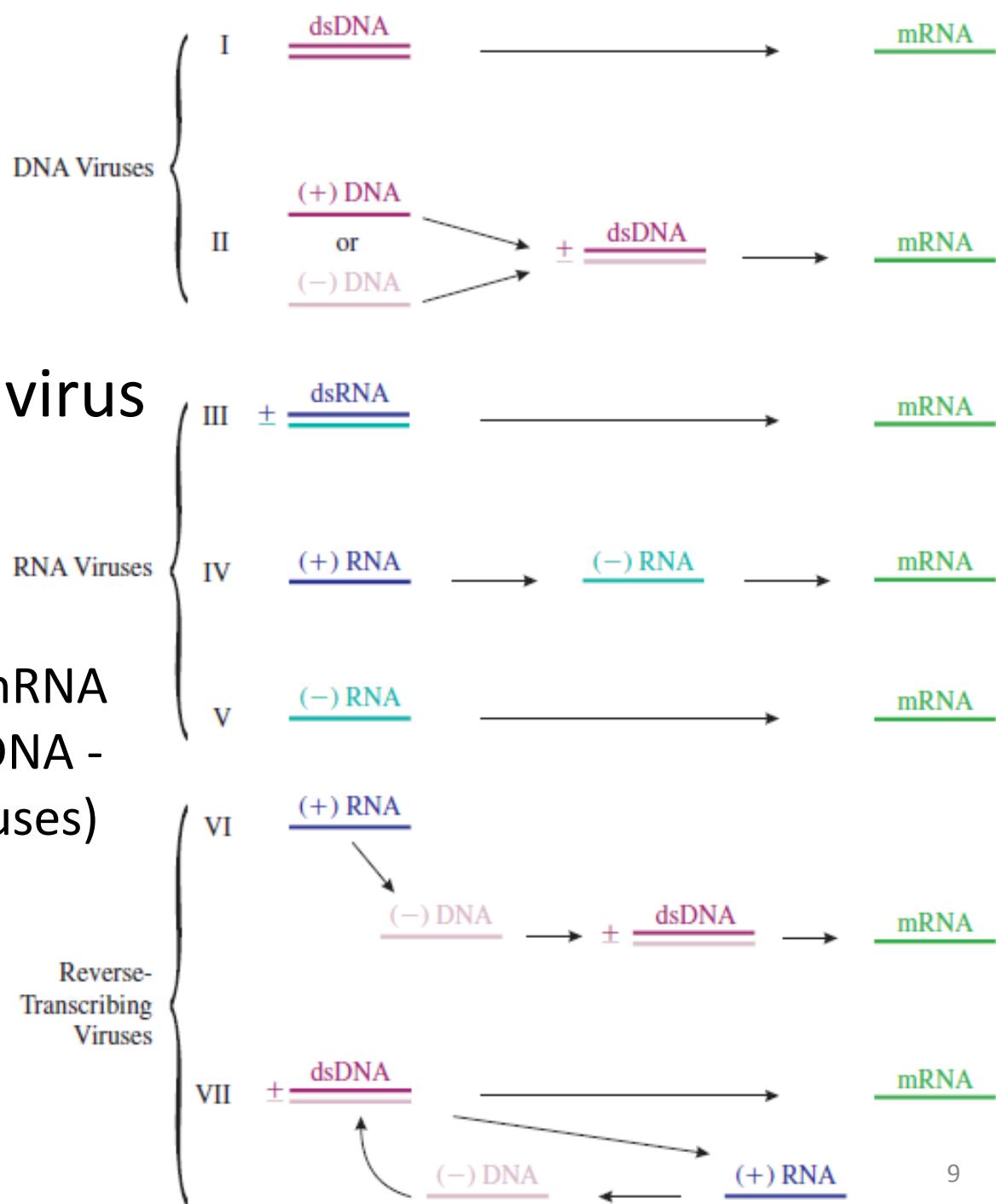
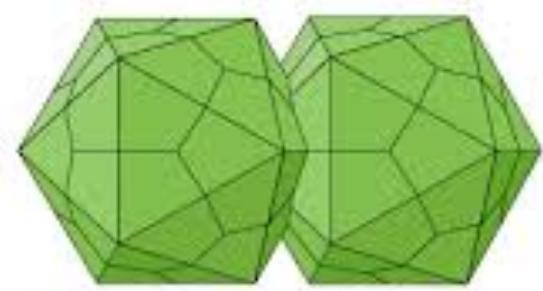


Post-Translational Modifications



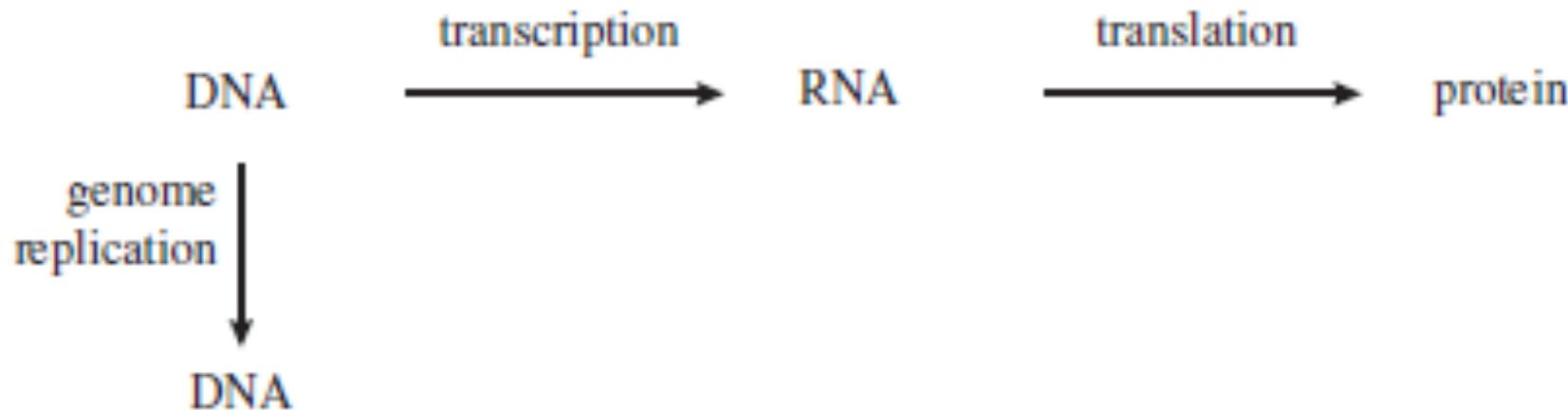
(David) Baltimore virus classification

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

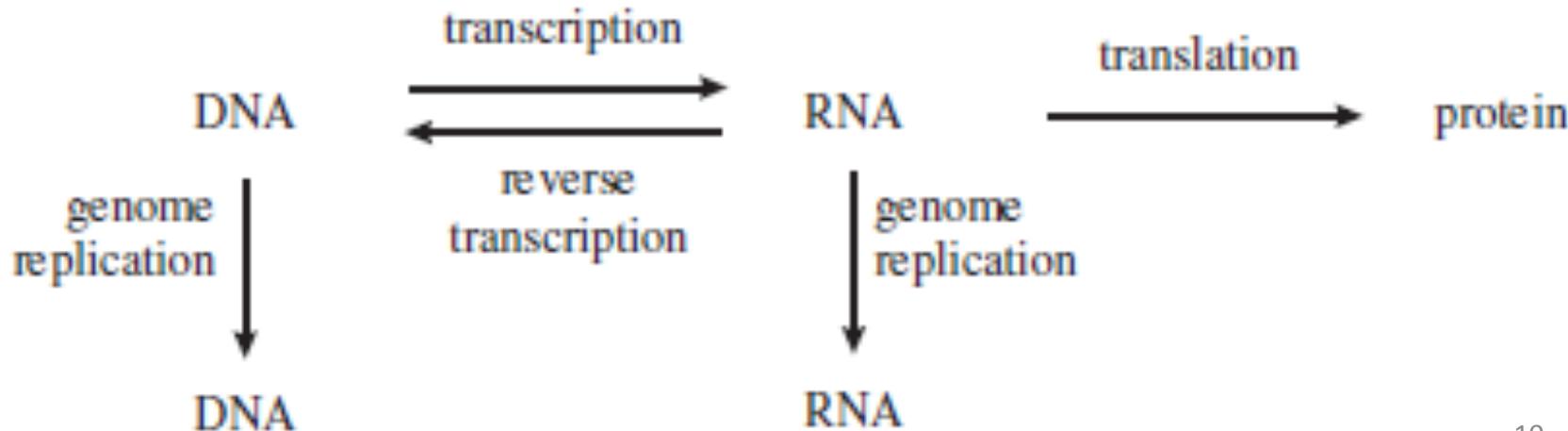


Central Dogma of Molecular Biology

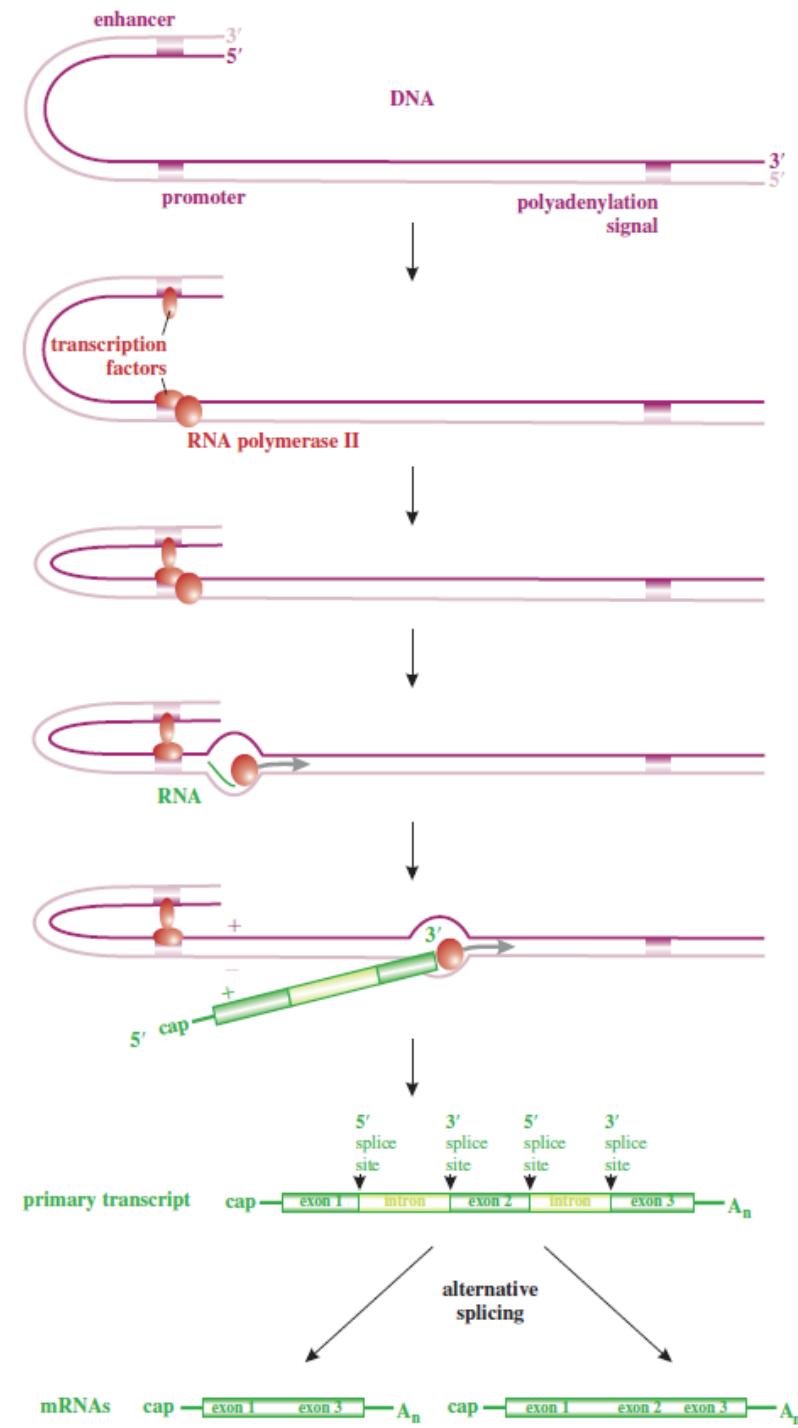
(a) Central Dogma



(b) Modified Central Dogma

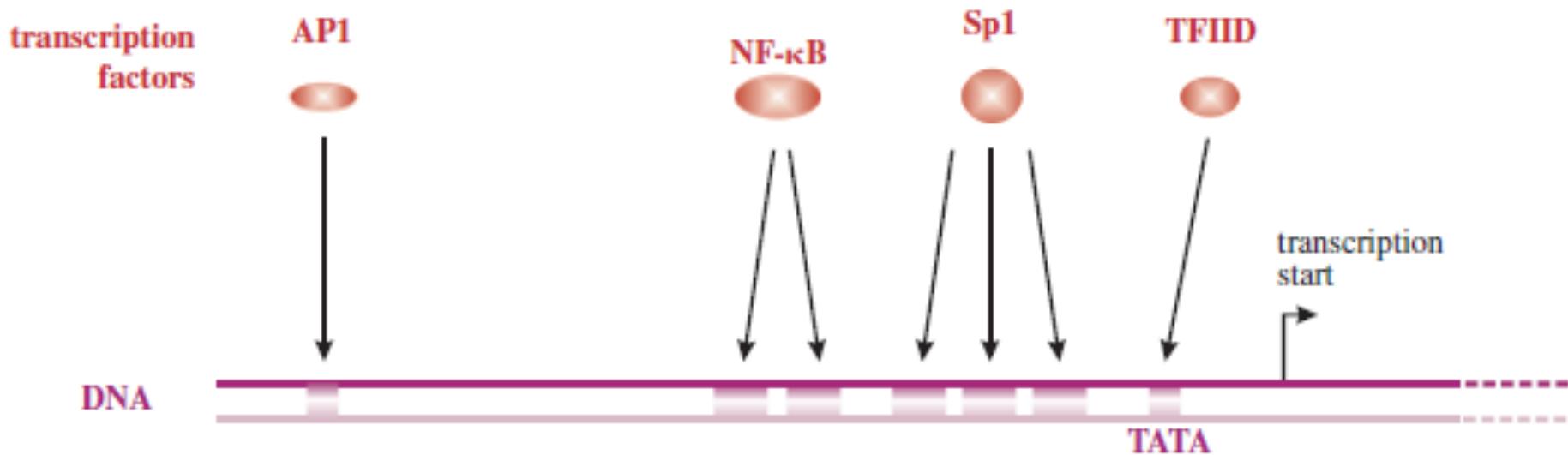


Transcription in Eukaryotes



Promoters, Enhancers, and TATA box

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



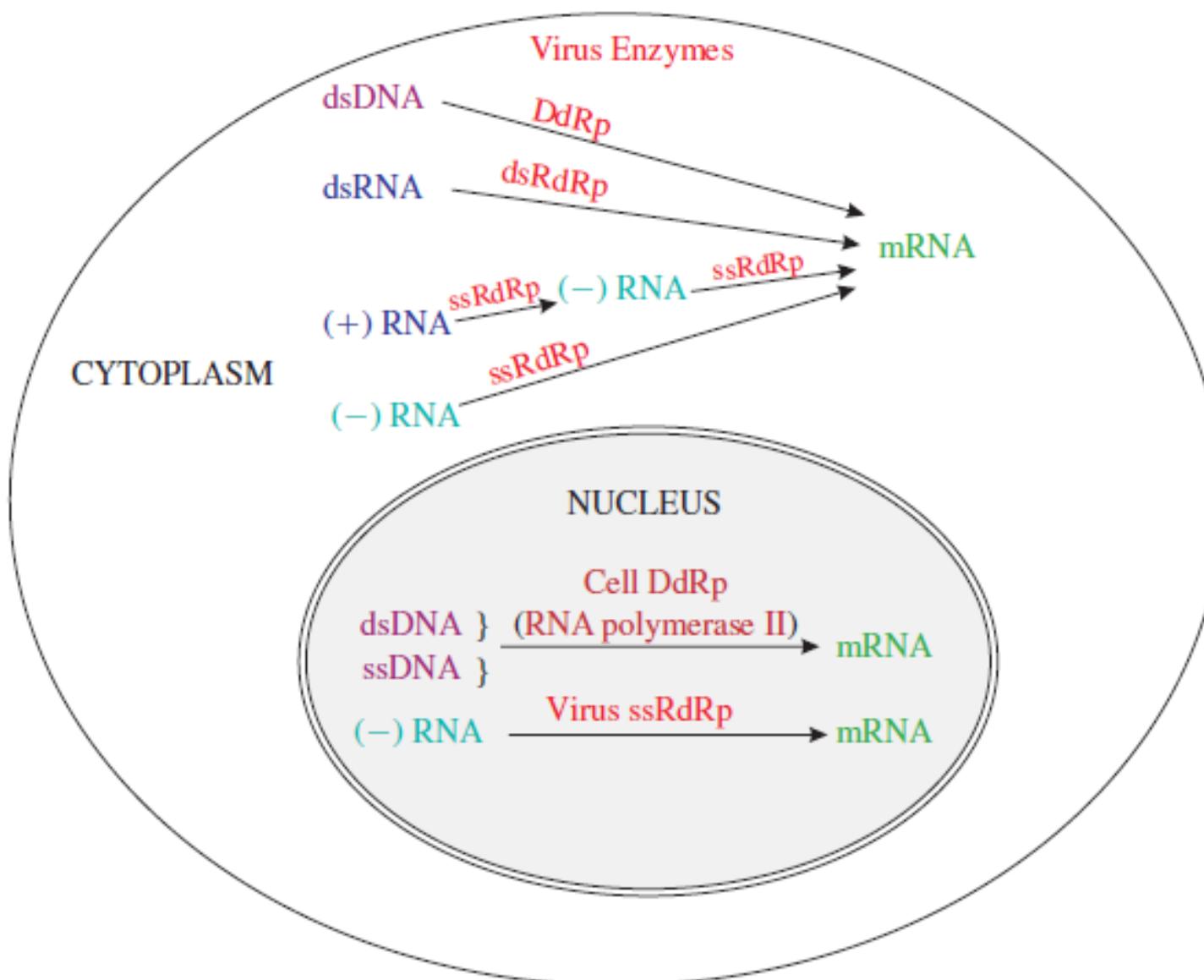
General cellular transcription factors: TFIID

Specific cellular transcription factors.

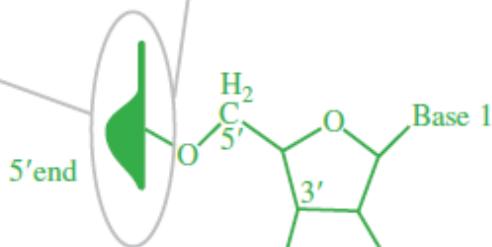
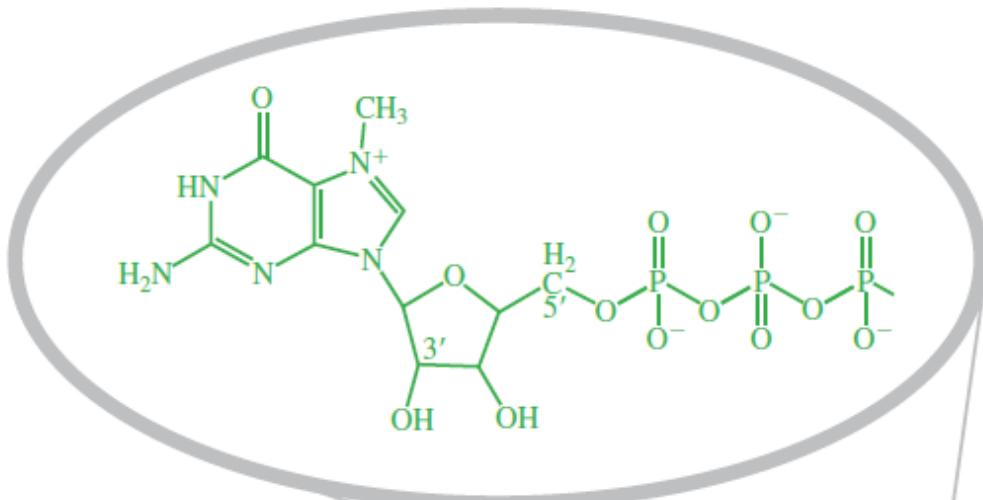
Virus transcription factors: VP16 of herpes simplex virus

- different transcription factors used during different stages of infection

Transcriptases



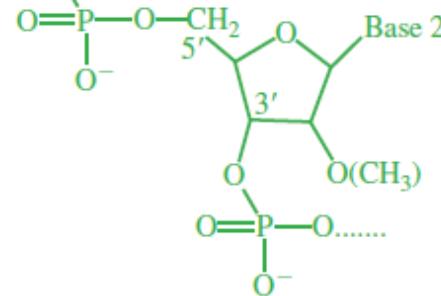
mRNA cap



Guanosine triphosphate
joined by 5'-5' linkage
(methylated in ribose)

- aid mRNA transport from the nucleus to the cytoplasm
- protect the mRNA from degradation by exonucleases
- be required for the initiation of translation

RNA triphosphatase
Guanylyl transferase
Methyl transferase(s)



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.

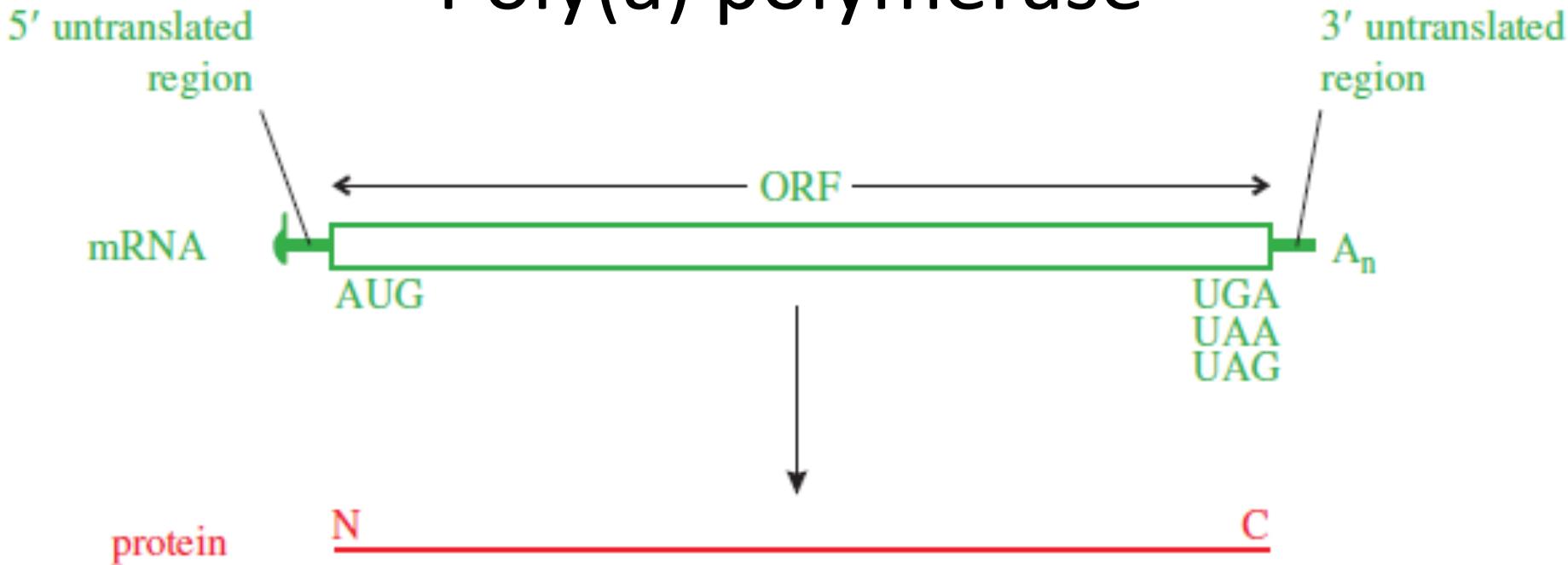
Cap snatching in cytoplasm – bunyaviruses

Non-capped mRNAs – picornaviruses

De-capping of host mRNAs – totiviruses

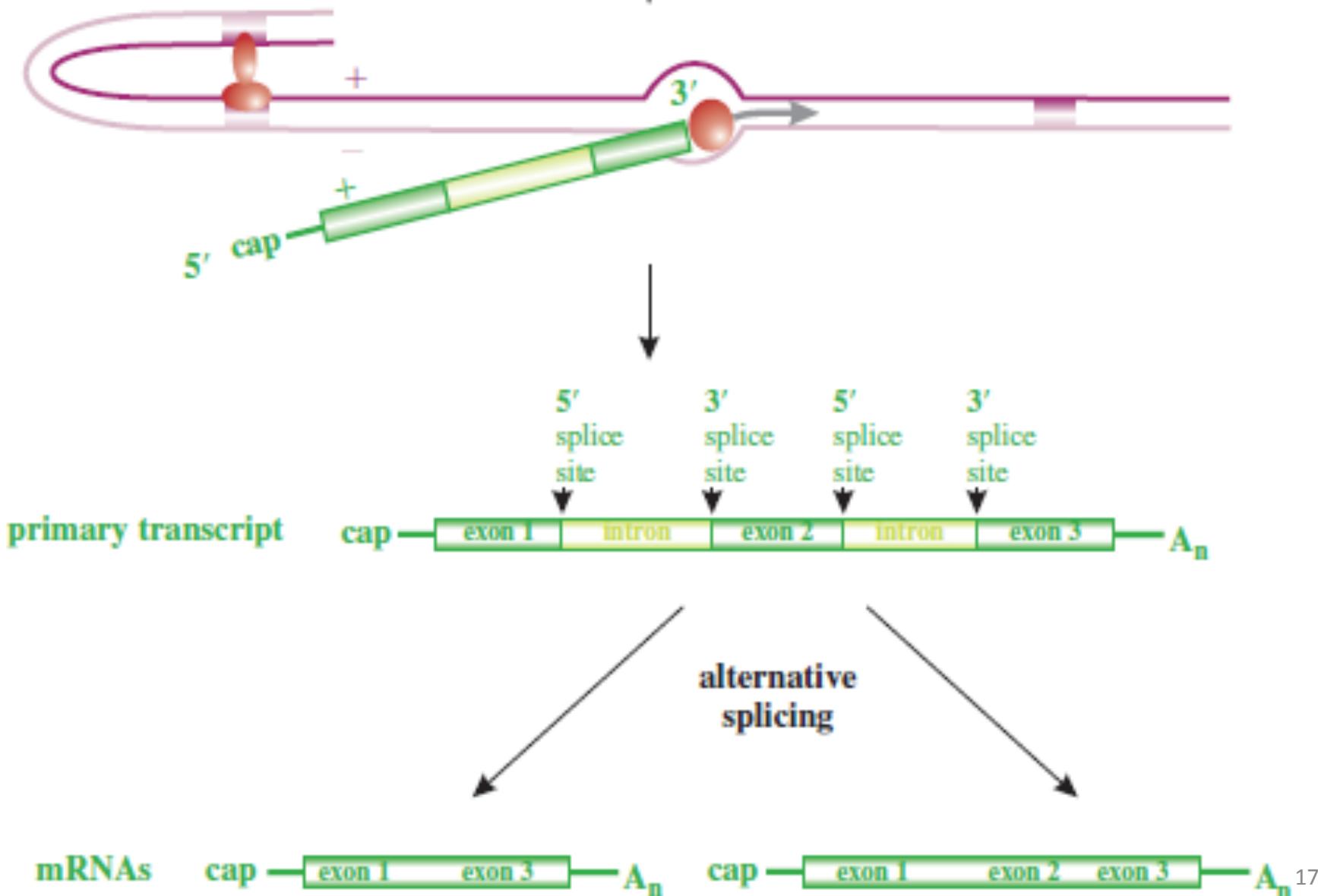
mRNA polyadenylation

A A T A A A
Poly(a) polymerase

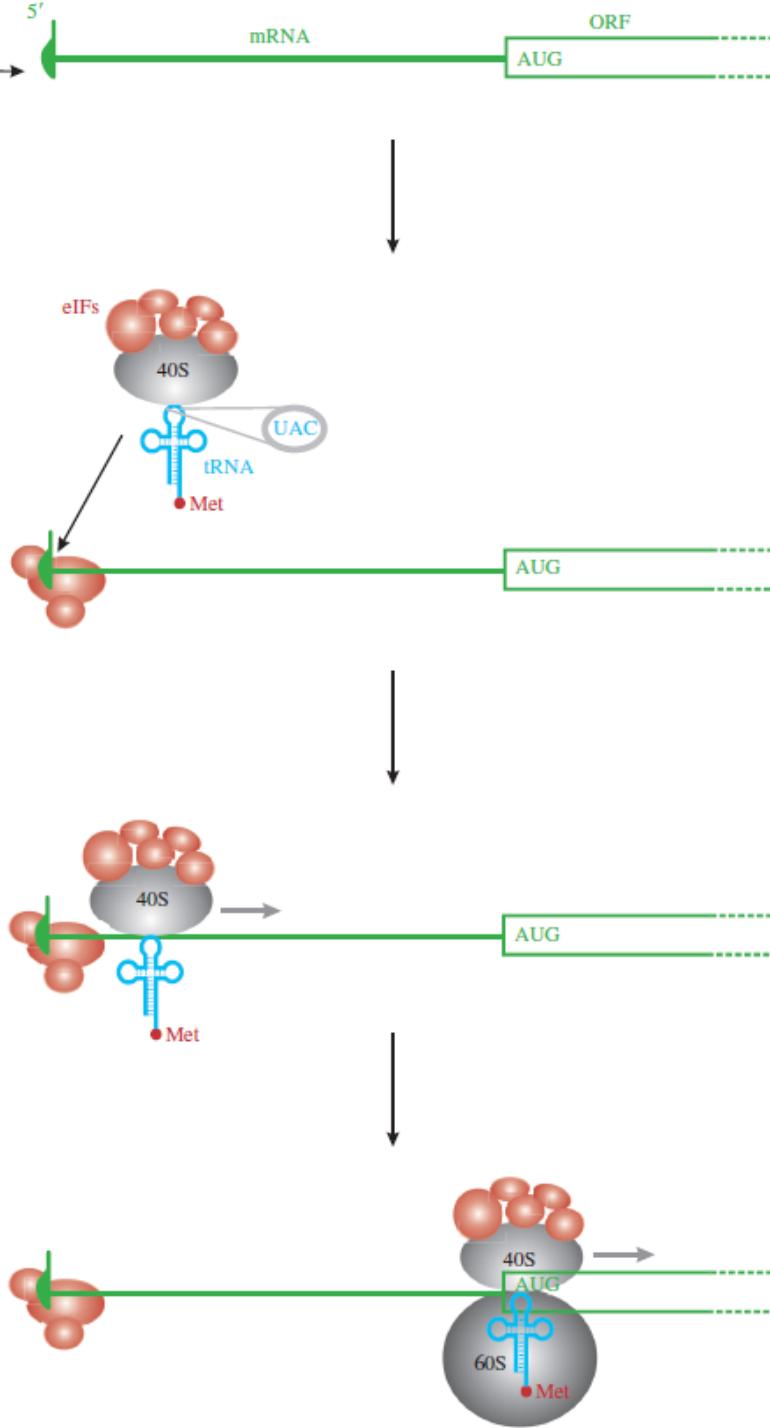


First identified in SV40 transcripts in 1981.

Pre-mRNA splicing

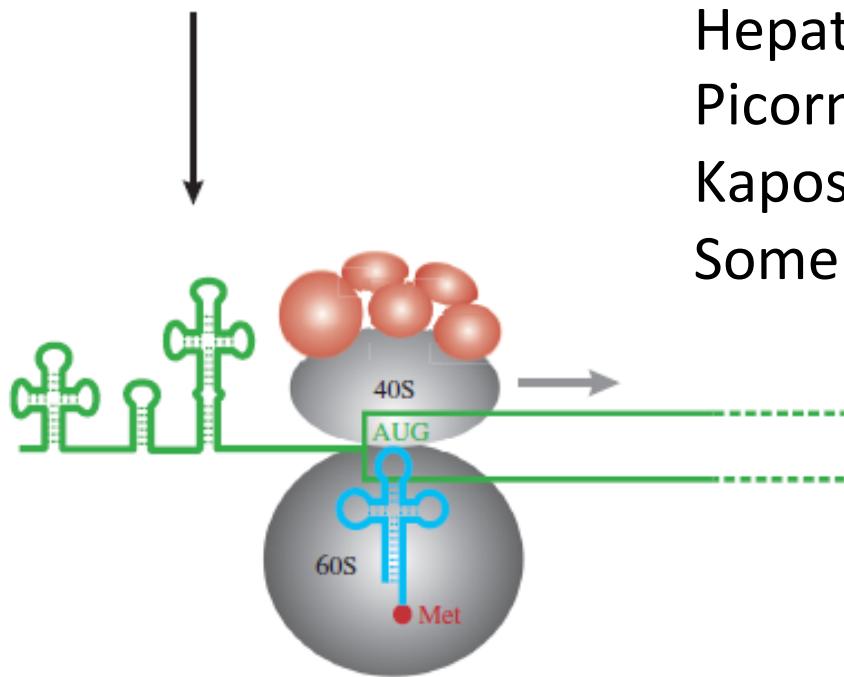
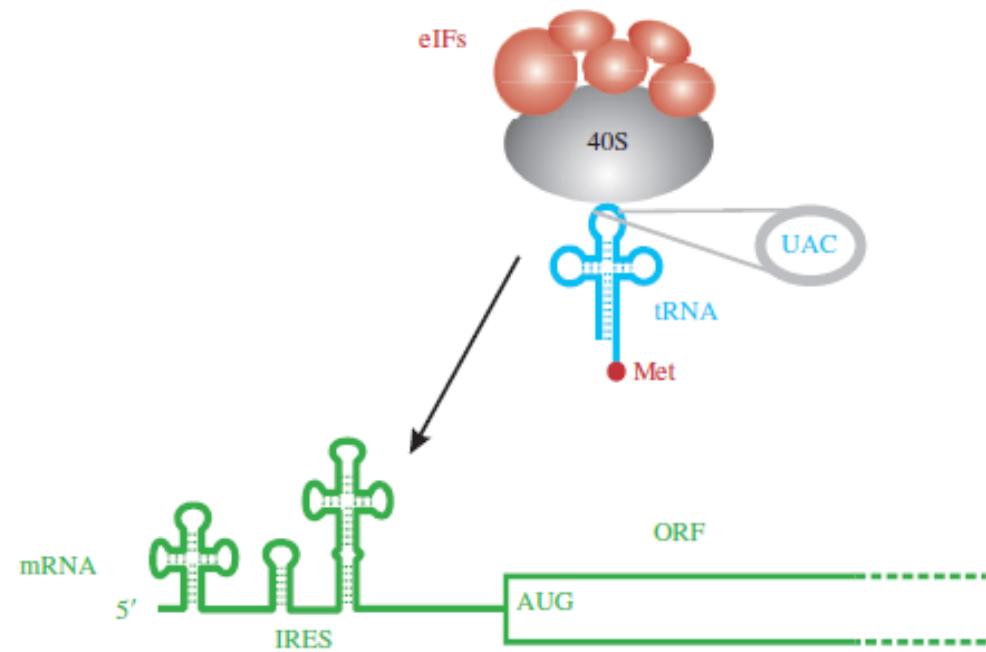


Translation

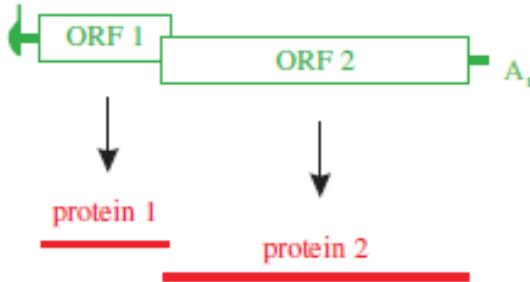


- Most viruses use host translation machinery.
- Mimiviruses encode their own tRNAs.
- eukaryotic Initiation Factors (eIF) (CAP)
- polyA binding proteins
- mRNA circularization
- 5'->3' scanning by 40S subunit (AUG) x Sendai virus (ACG)
- IRES

IRES dependent Translation



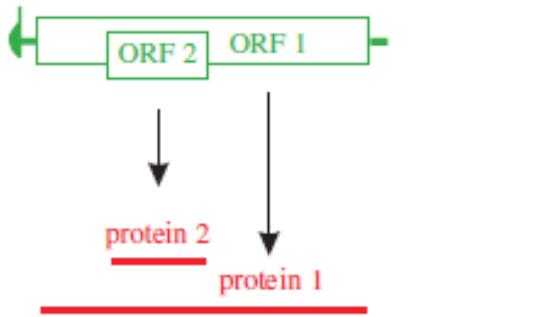
Hepatitis C virus
Picornaviruses
Kaposi's sarcoma associated herpesvirus
Some cellular mRNAs



Leaky scanning

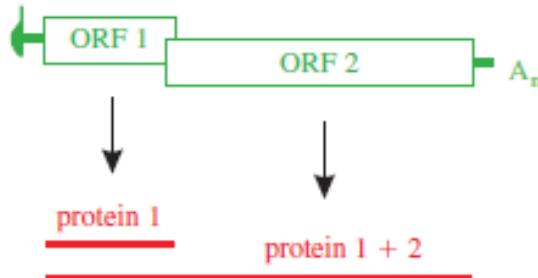
HIV-1 Vpu and Env
(Chapter 18)

Bicistronic mRNAs



Leaky scanning

Rotavirus NSP5 and NSP6
(Chapter 13)

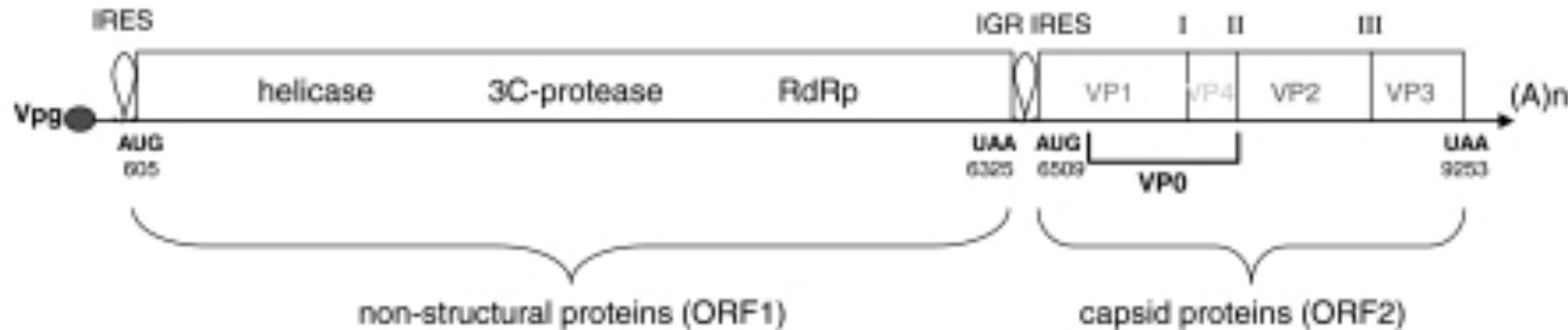


Ribosomal frameshift

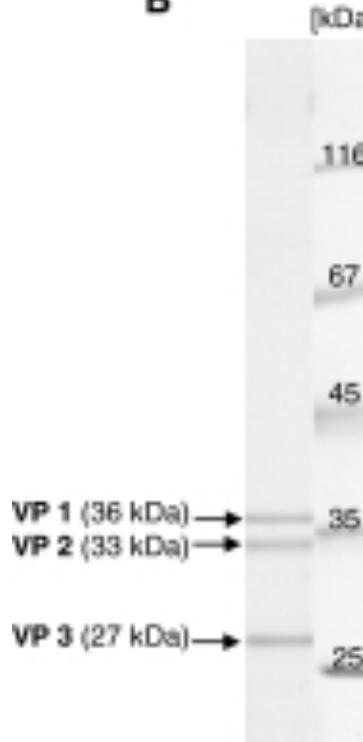
HIV-1 Gag and Gag-Pol
(Chapter 18)

Bicistronic mRNAs of dicistroviruses

A



B



C

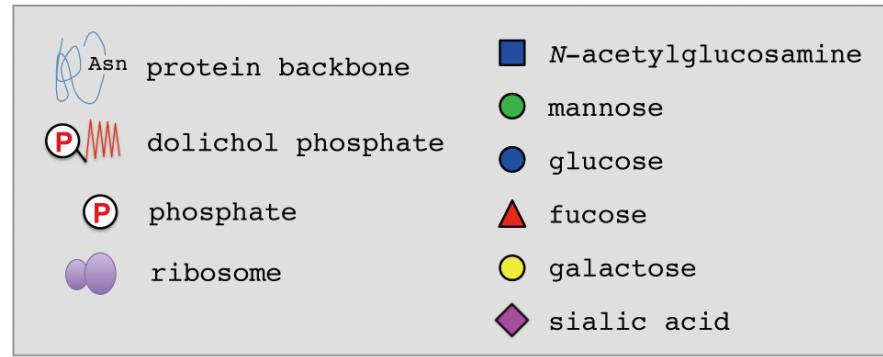
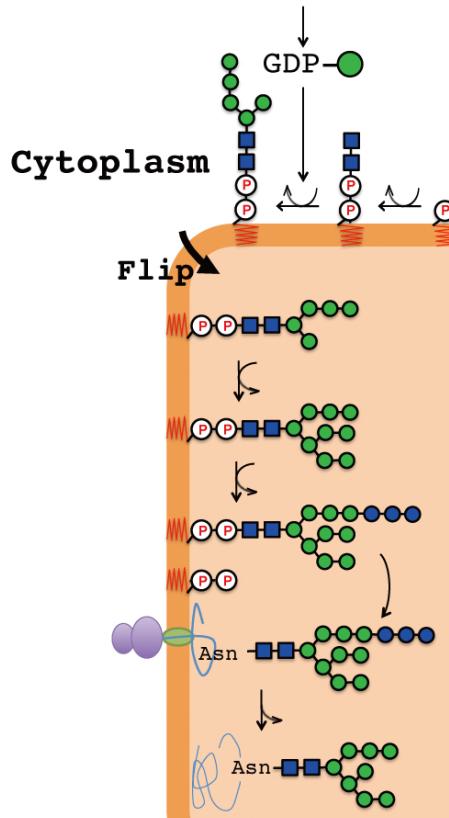
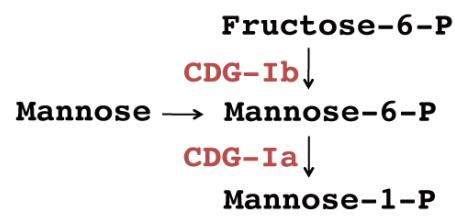


D

1 MHAIAFRNTIP ADQETNTNSNV HNTQLASTSE ENSVETEQIT TFIHDEVETPWR
 51 IDTPMNAQDTS RARSMDOTHS IIQFLQRPVLP IDHIEVIAGS TADONKPLNHR
 101 YVLNWQRNPQP FVRSTWTLPSV VLSAGGEGGCK LANFKYLRCID VRVKIVLNMN
 151 PFIAGIRLYLA YSPYDDDRVDP ARSILNTSRA GUTGYPGEVEI DFQLDNSVEM
 201 TIPYASPGQA YDLVTGTTEDP VKLYLPTITP IL8PTSTBAS SKVDSLVYMN
 251 LDHISLVIPT YRVNNTSIVPH VRTVYVQAVQN ITTRDGETIR KAMVALRENN
 301 KSTYDYIVQKA L88AVPEVKH VTMQIN8KCNH NPNCMATSVK EKPK**SILKPK**
 351 TENPKIGPI8 ELATQVHKVA HGLERIPVIG EMAKPVBTI KHVADKIQSYV
 401 AAIPK**WSKPR** NLEQVNLYQN VPOWQYSLYK QIDNSVPLAP DPNEELQDLR
 451 DVPPSGQVDEM AIGYVQGNPA VKHVL8WNNT DKVQVPIBNG DDWQGVIPVG
 501 MPCYSKIIINT TENETTQTET EVMDPAPCEY VCNMPBSYWRH TMCYRIAIAVK
 551 TAFHTGMRLEI FPEPMGRIPIT TTKDNISPDL TQLDGIKAPS DNNYKYILDL
 601 TMDTEKITIRV PFVSNKMFMK STGIYQGNSE NNWDPFBESPT GFLCIRPVTK
 651 LMCPETVSNN VSIVVWENAE DVVVVEPKPL LSGPTQVFQF PVTBADSINI
 701 IDAS*MQINLA* MKADENVITF FDSDHAKERN MEALLKGSQE QIMNLRLLLA
 751 TFRTISENWN LFPFTKTAIT DLTDVADKEG RDYMSYLSYI YRFYRGGRRY
 801 KFFMHTTALEQ SGTCYIIRSFL VPRYYTTDNT KNDGFSHITY PVLMFVHEVE
 851 VPYYCQYRKL PVASTTDQGY DASLMYYSQNV GTNQIVARAG HDOOTFGWLI
 901 GTPGTQGIGR TETK

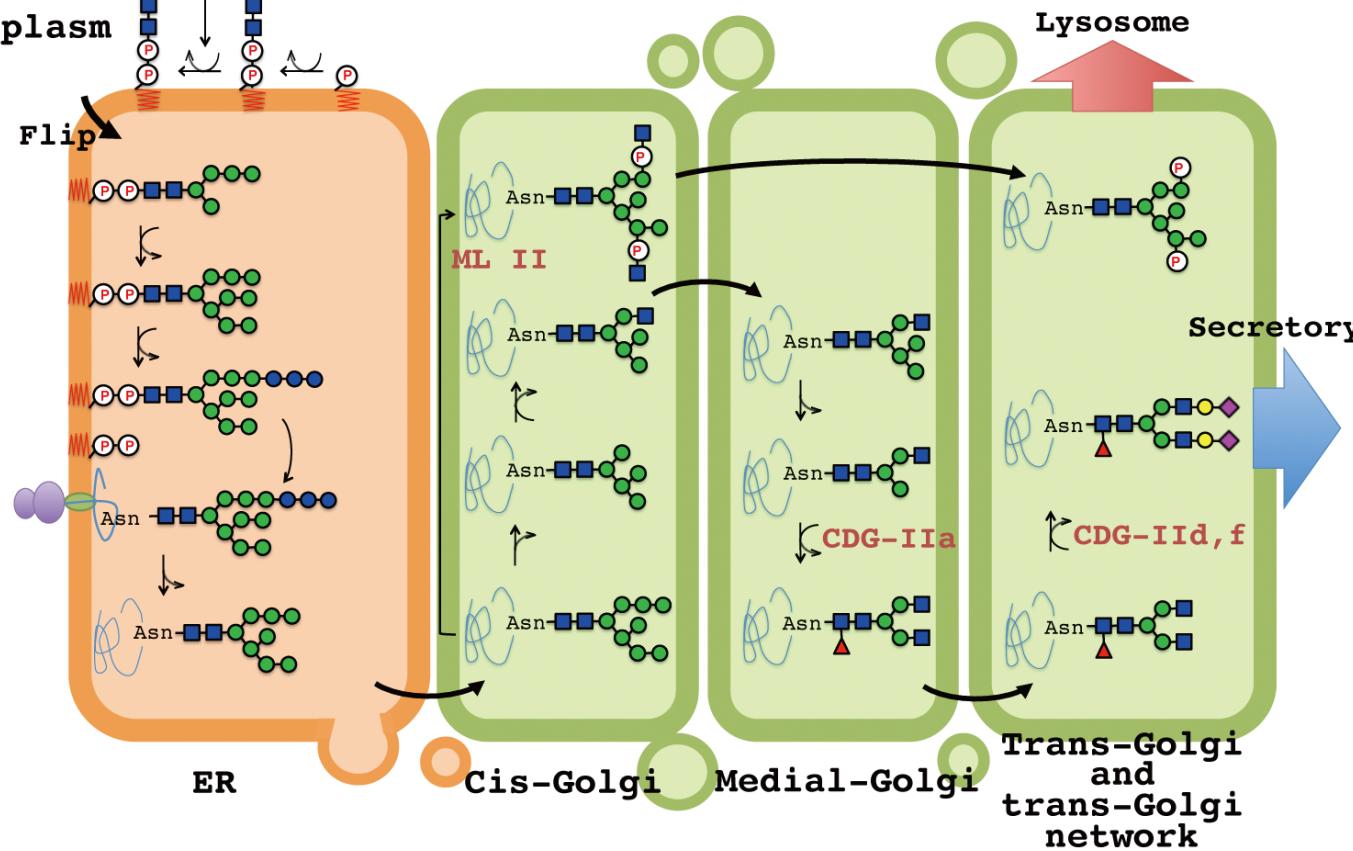
VP 1
VP 4
VP 2
VP 3

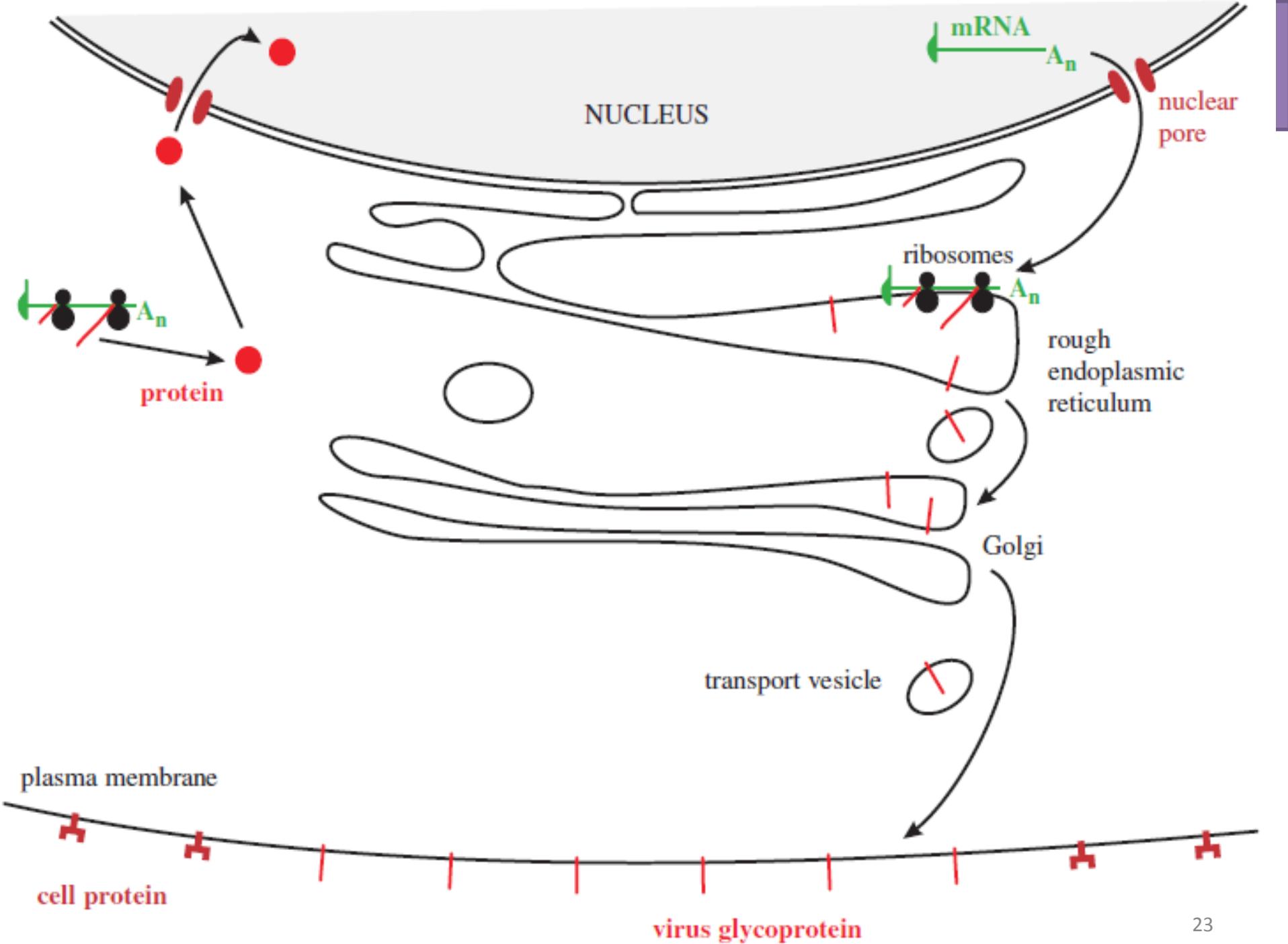
Co- and post translational protein modification



N-glycosylation
(Asn)

O-glycosylation
(Tyr, Ser)





Co- and post translational protein modification

Acylation (addition of Myristic acid)

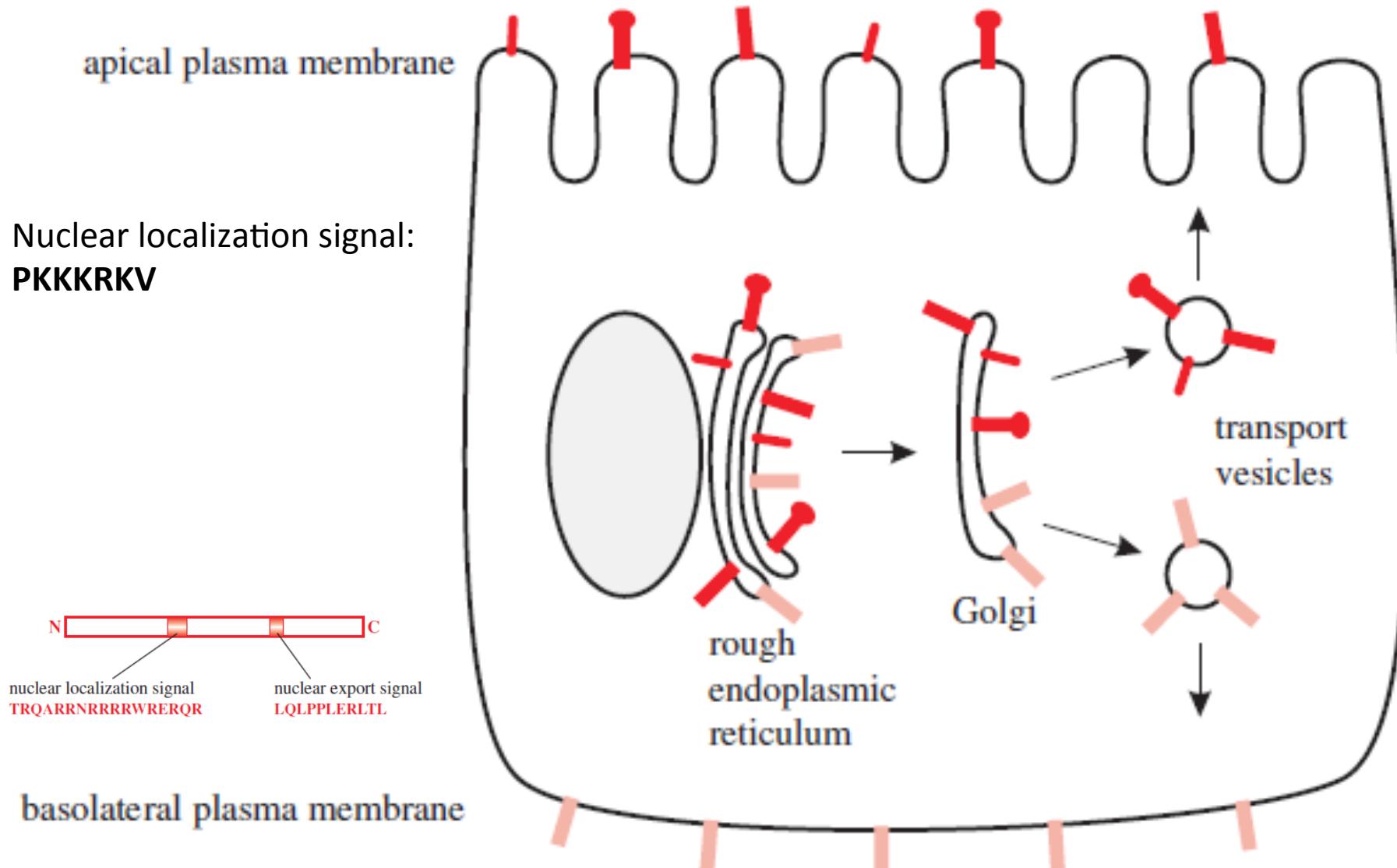
- Gag of HIV
- VP4 of picornaviruses

Phosphorylation

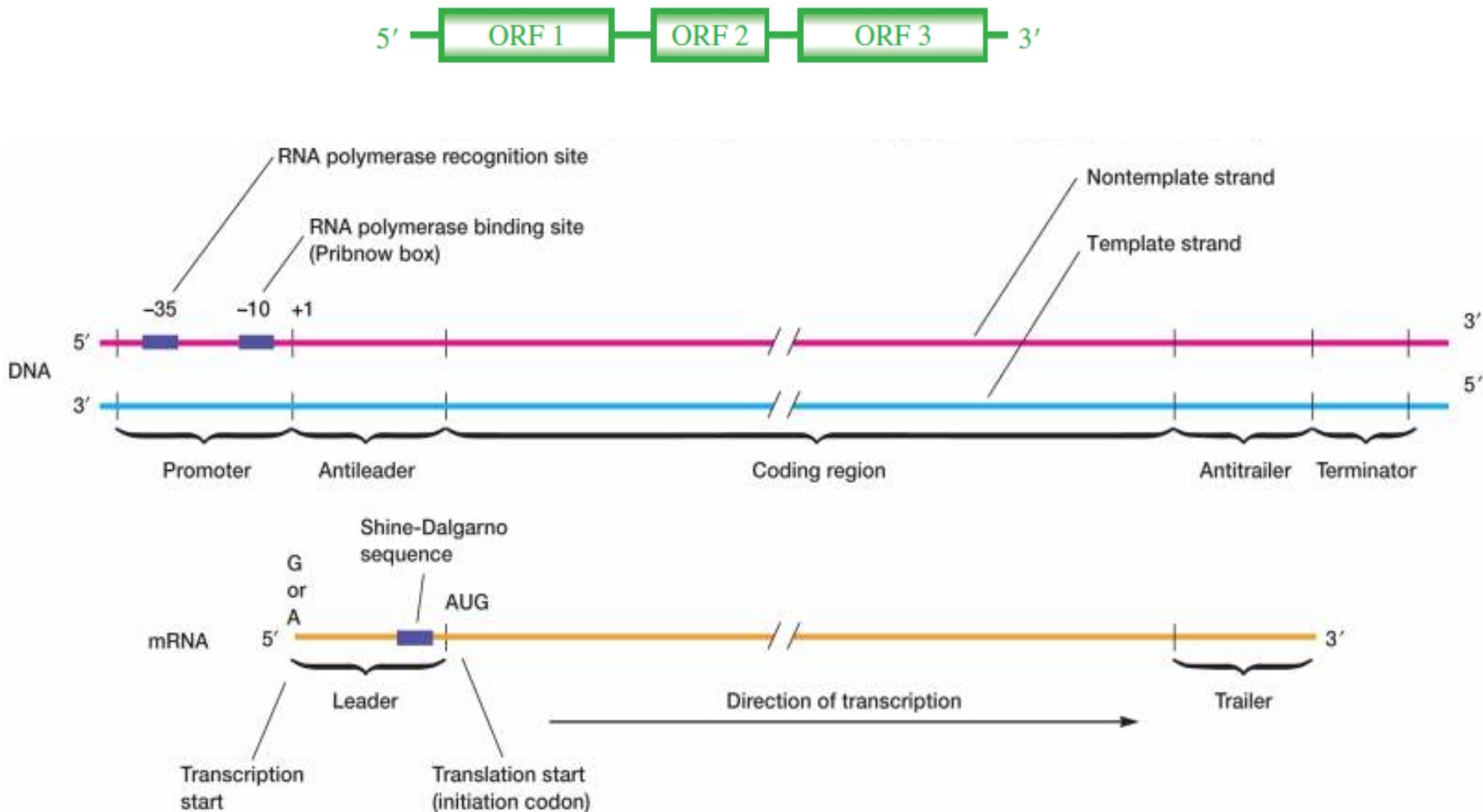
- serine, threonine, tyrosine

Cleavage

Targeting of virus proteins



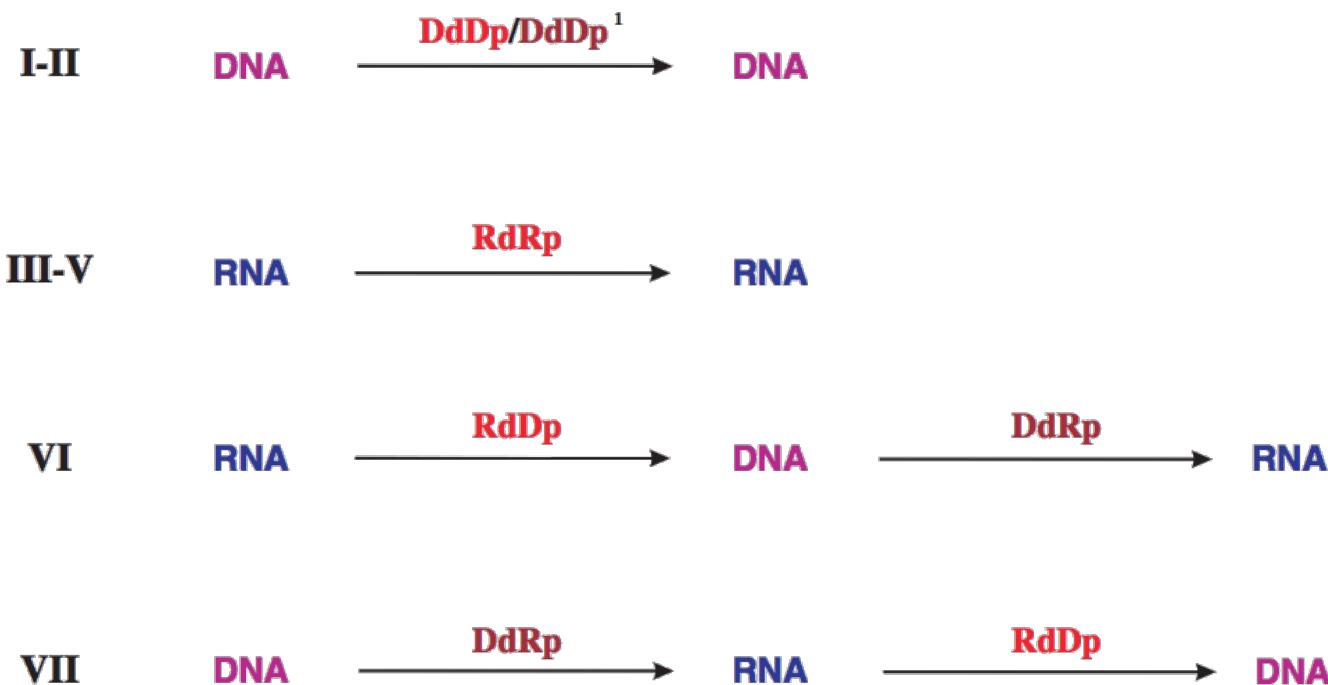
Transcription in bacteria



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

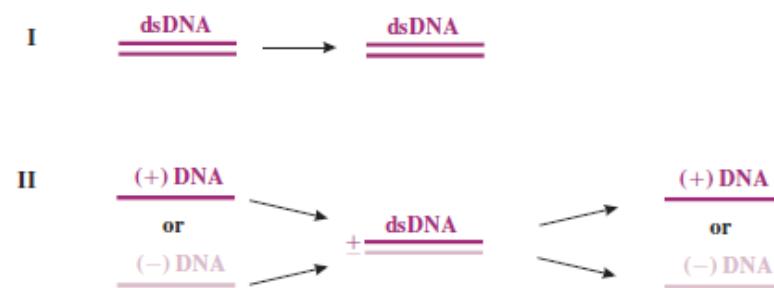
NUCLEUS

some dsDNA viruses
ssDNA viruses
(-) RNA viruses (segmented genomes)
retroviruses & pararetroviruses (DNA → RNA)

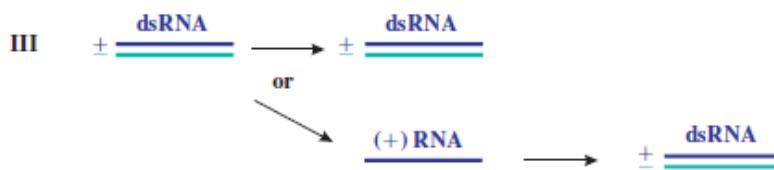
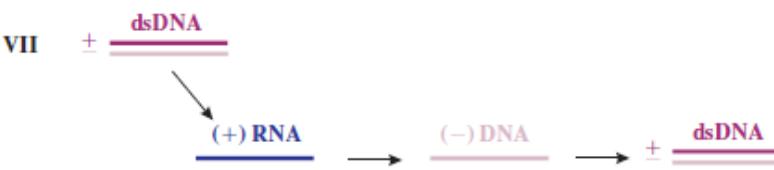
Primers



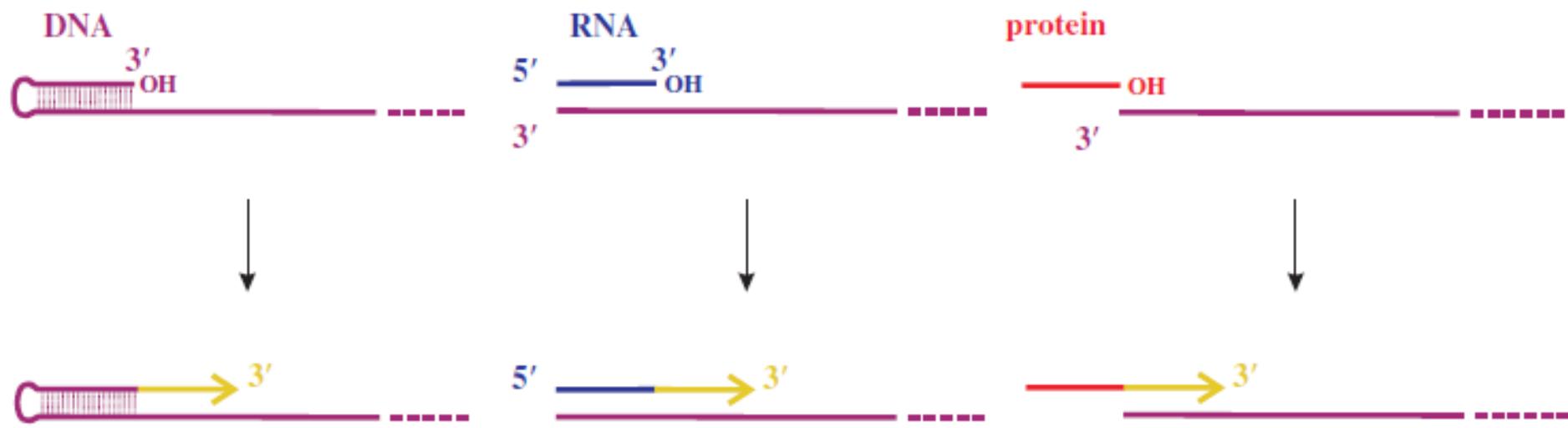
DNA Viruses



RNA Viruses

Reverse-Transcribing
Viruses

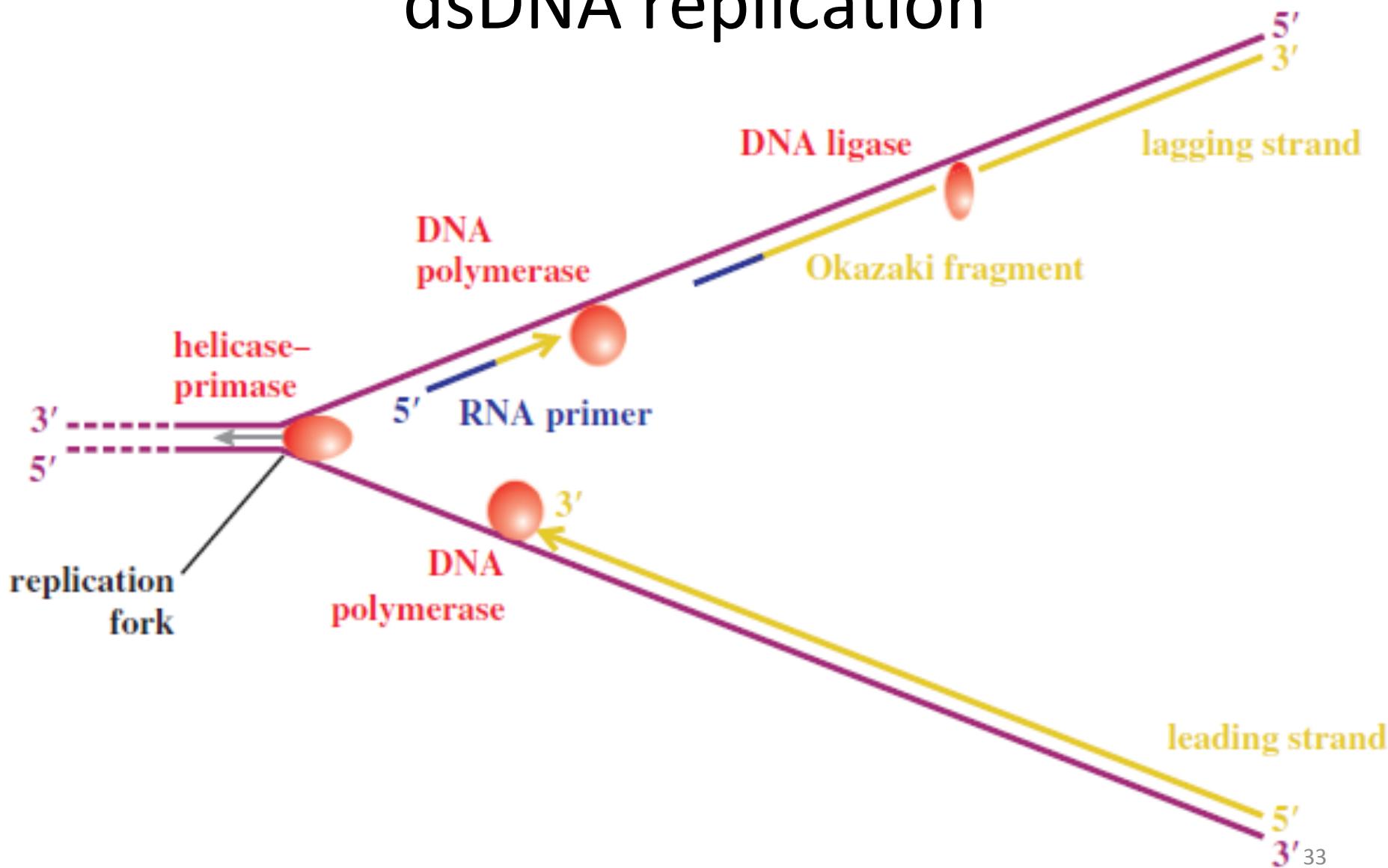
Priming of NA synthesis



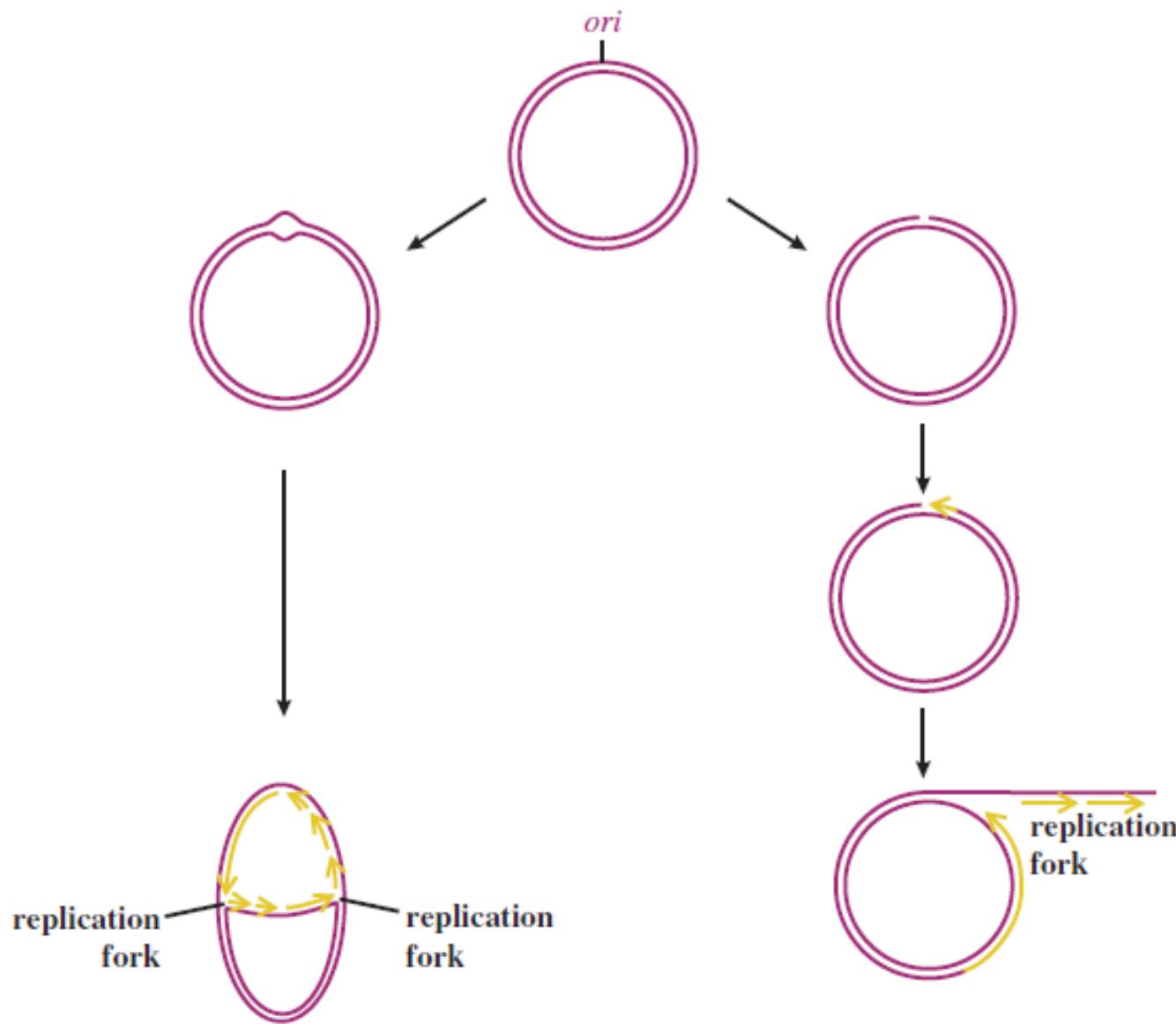
Location of virus replication sites in eucaryotes

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

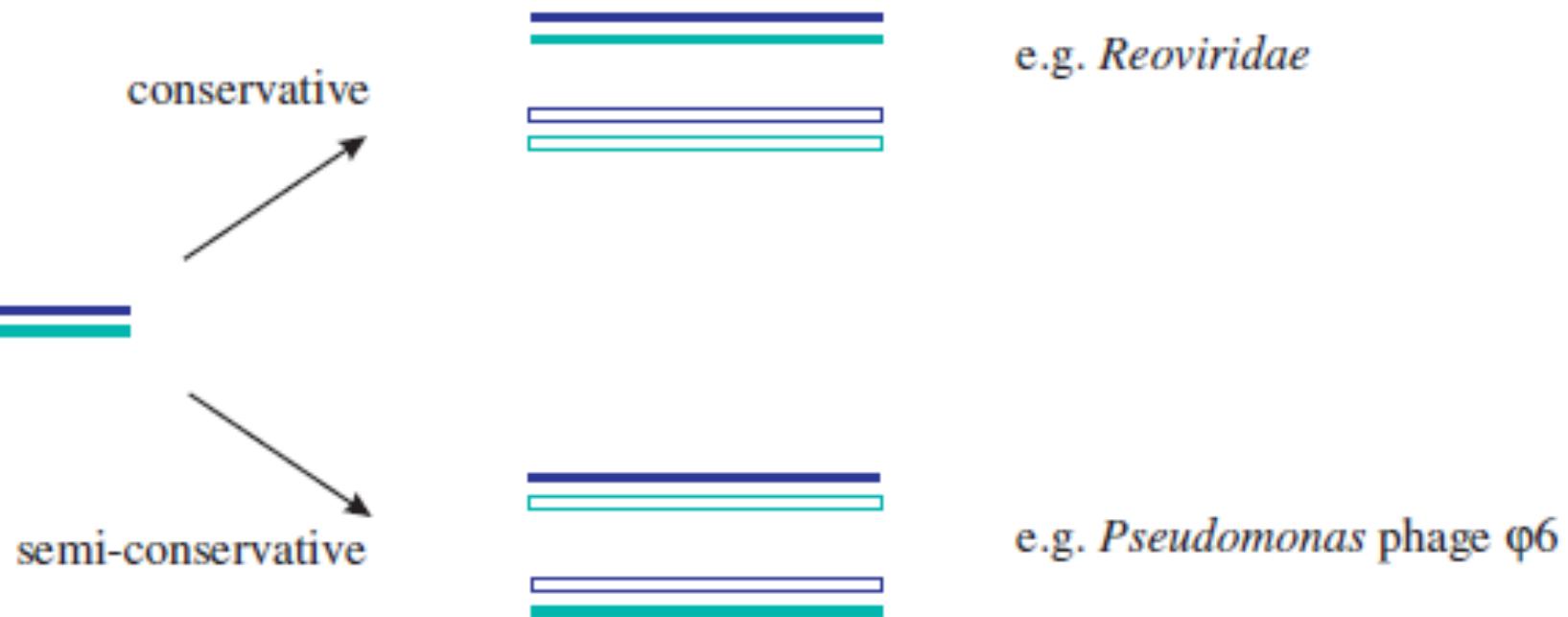
Leading and Lagging strands in dsDNA replication



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’