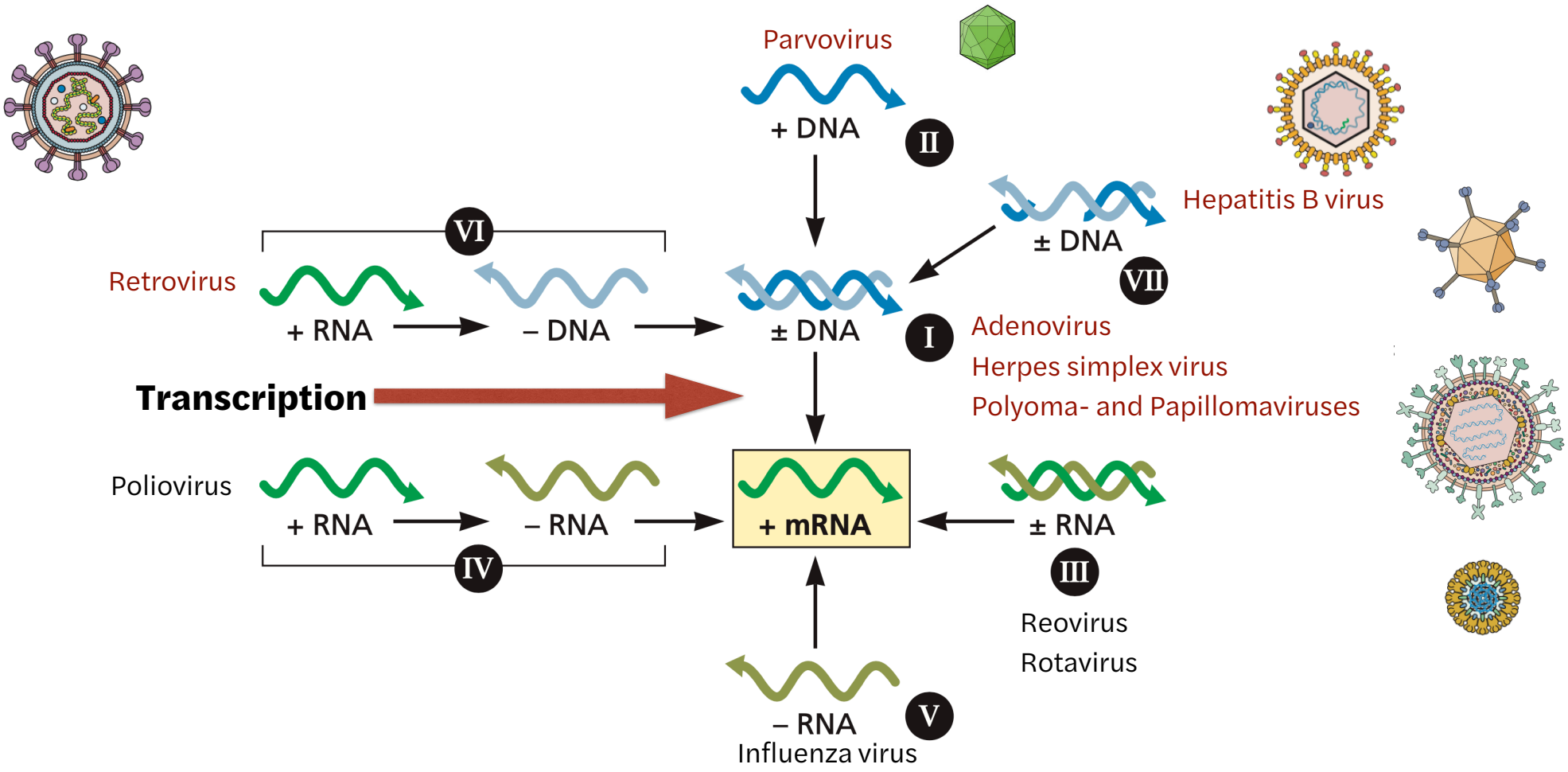
Transcription and RNA processing

Session 7

Virology Live

Fall 2021

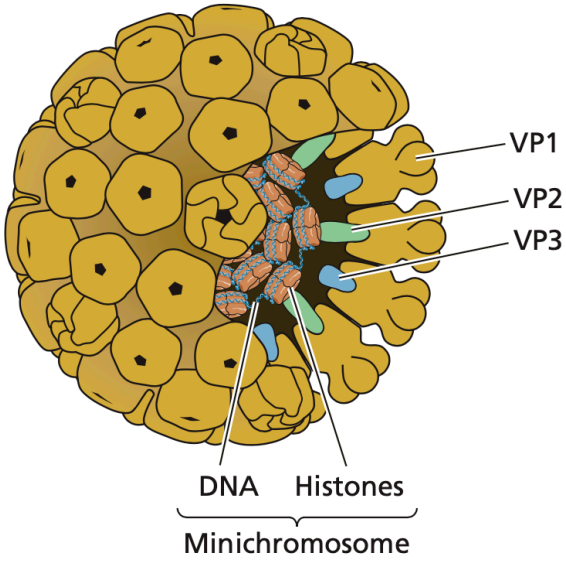
None loves the messenger who brings bad news
—Sophocles

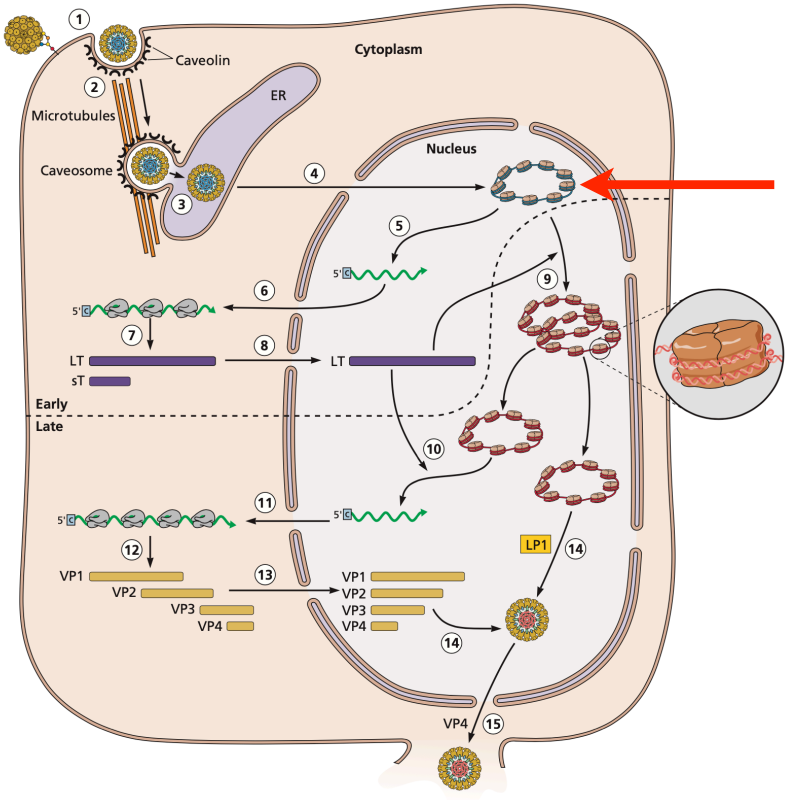


In common to all the viruses in red: they have dsDNA in their reproductive cycles

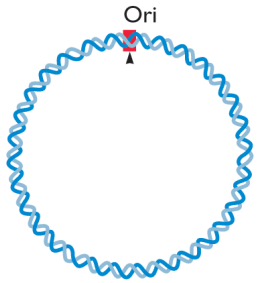
In cells infected with DNA viruses, at least one protein, often many, are needed for DNA replication

But not all DNA templates are ready for transcription!

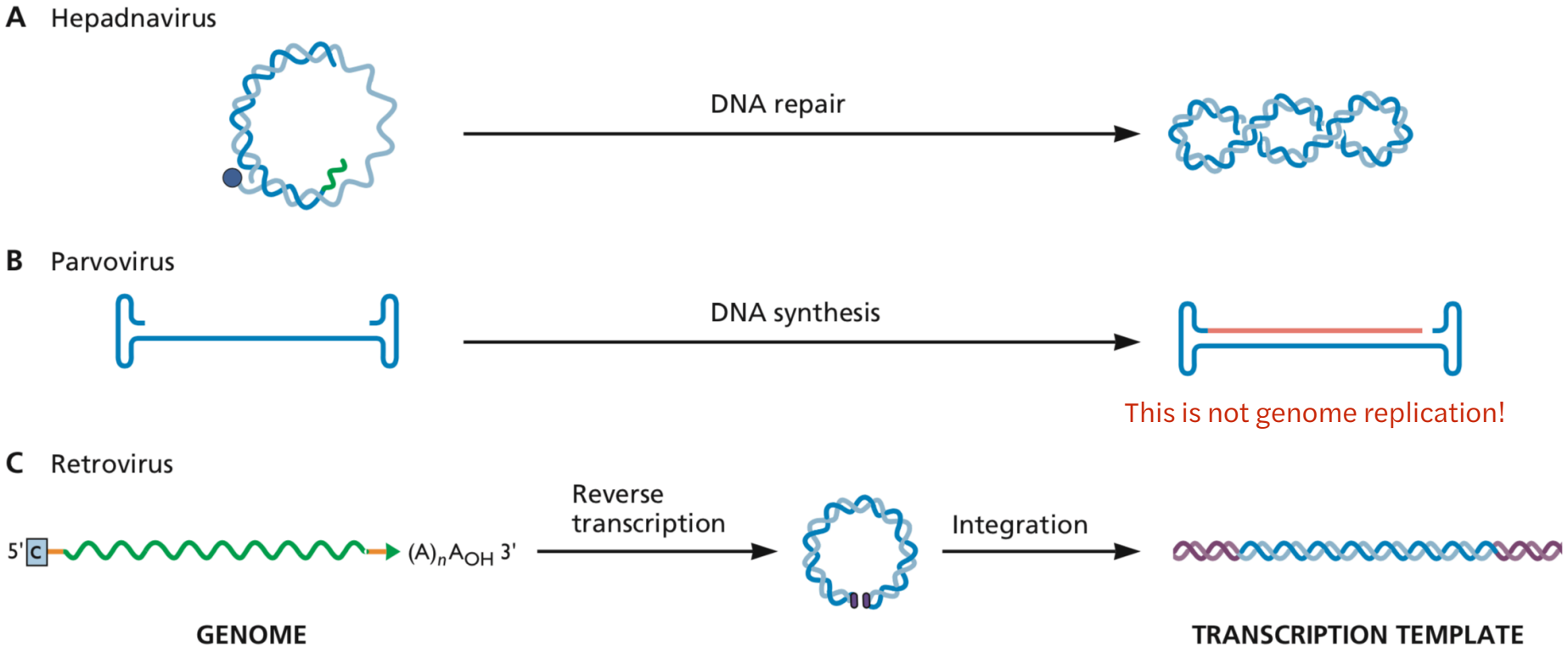




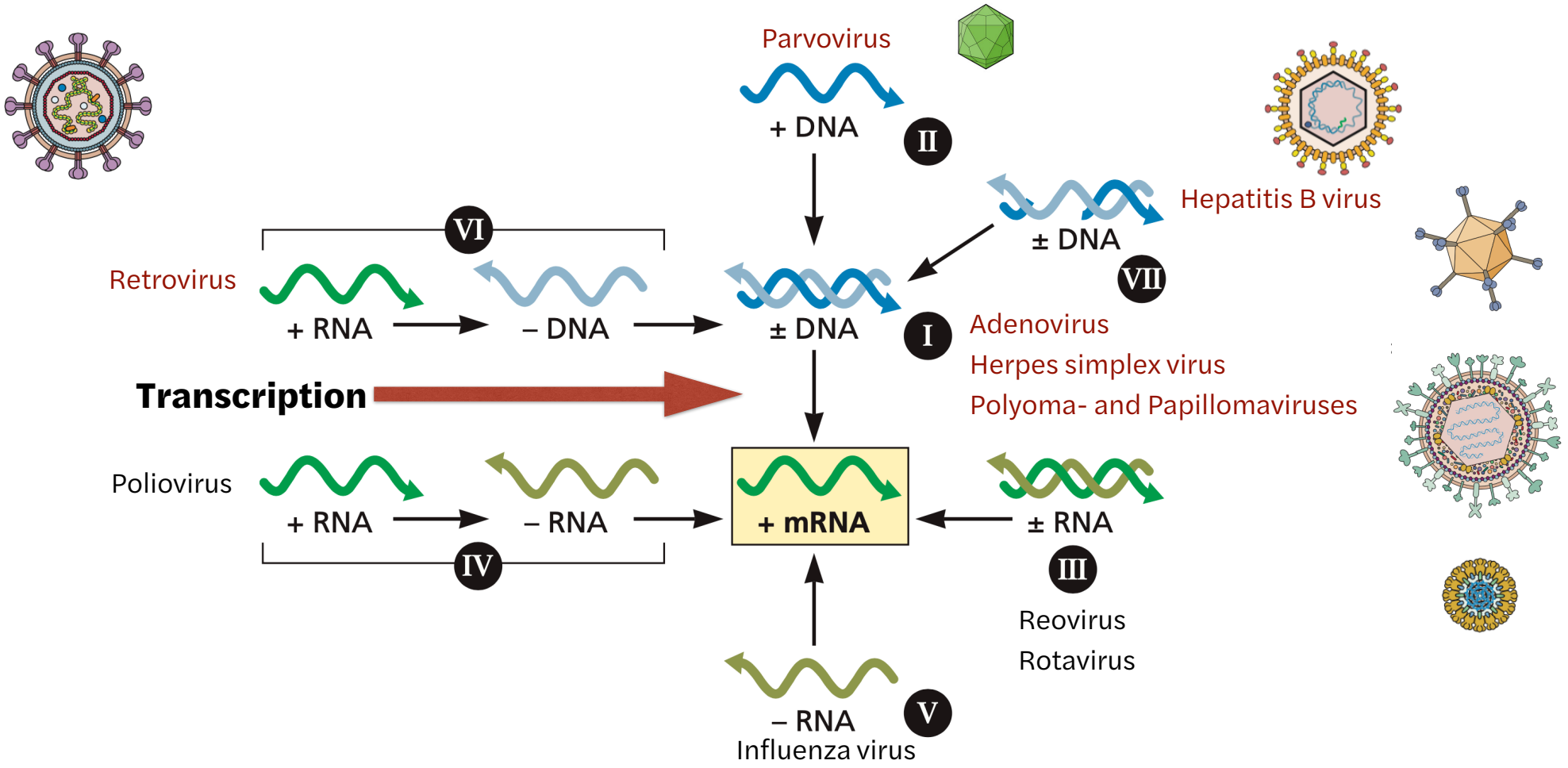
This one is ready!



Some viral DNA genomes must first be converted to templates for transcription



Which viral genomes do not need conversion?

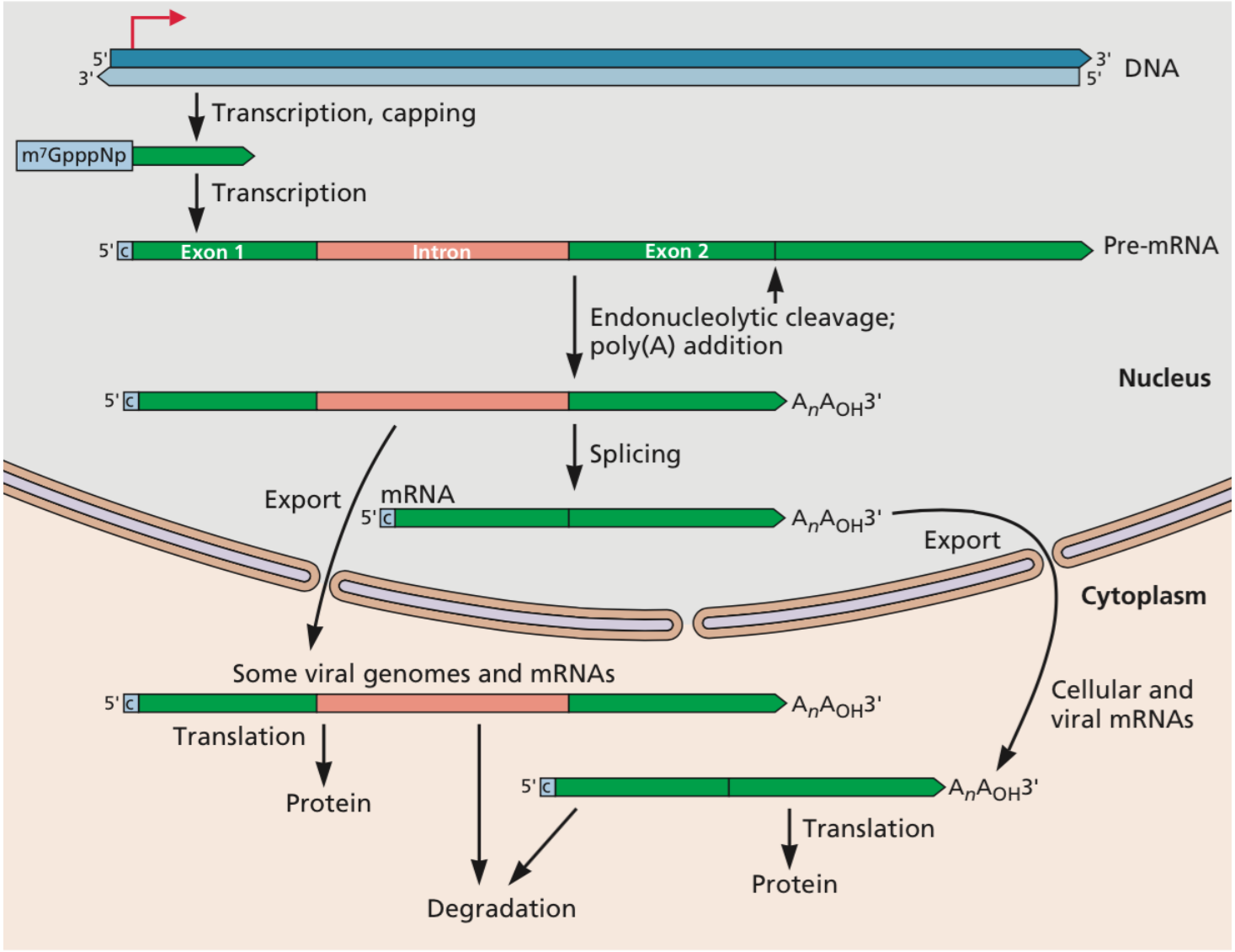


Eukaryotic DNA-dependent RNA polymerases

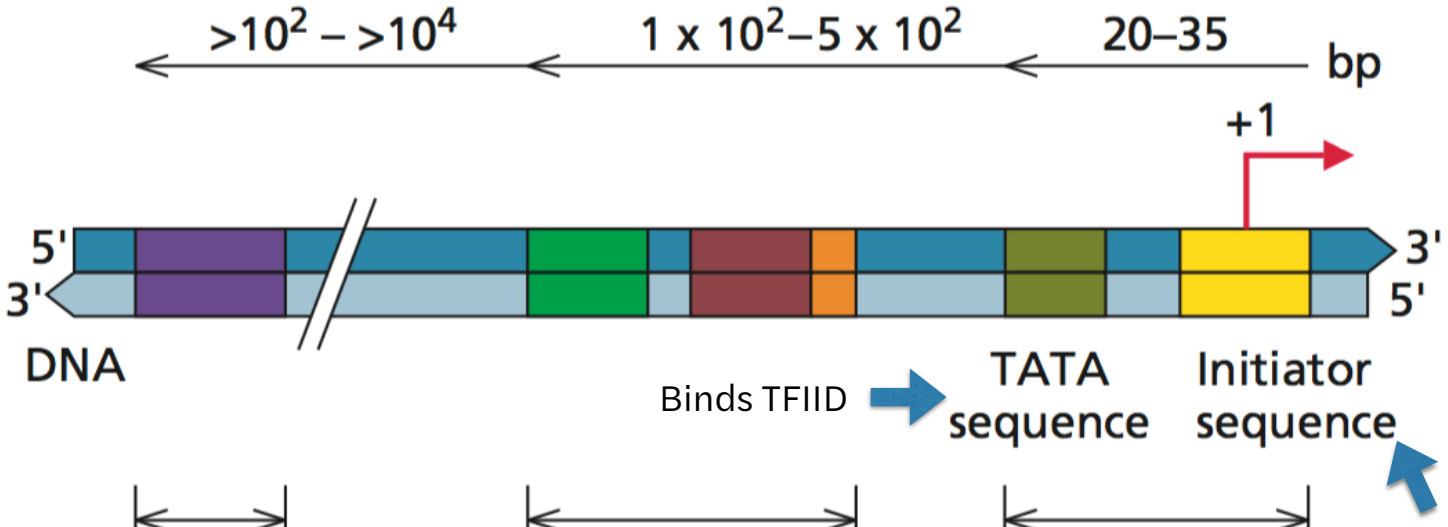
All initiate RNA synthesis de novo (no primer needed)

Enzyme	Cellular RNA	Viral RNA
RNA pol I	Pre-rRNA	None known
RNA pol II	Pre-mRNA Pri-miRNA SnRNA LncRNA	Pre-mRNA Pri-miRNA HDV genome RNA and mRNA HHV8 PAN RNA
RNA pol III	Pre-tRNAs 5S rRNA U6 snRNA	Ad-2 VA RNAs HBoV1 Boca SR MHV68 pri-miRNA

Only DNA viruses that replicate in cytoplasm (poxvirus, giant viruses) encode an RNA pol

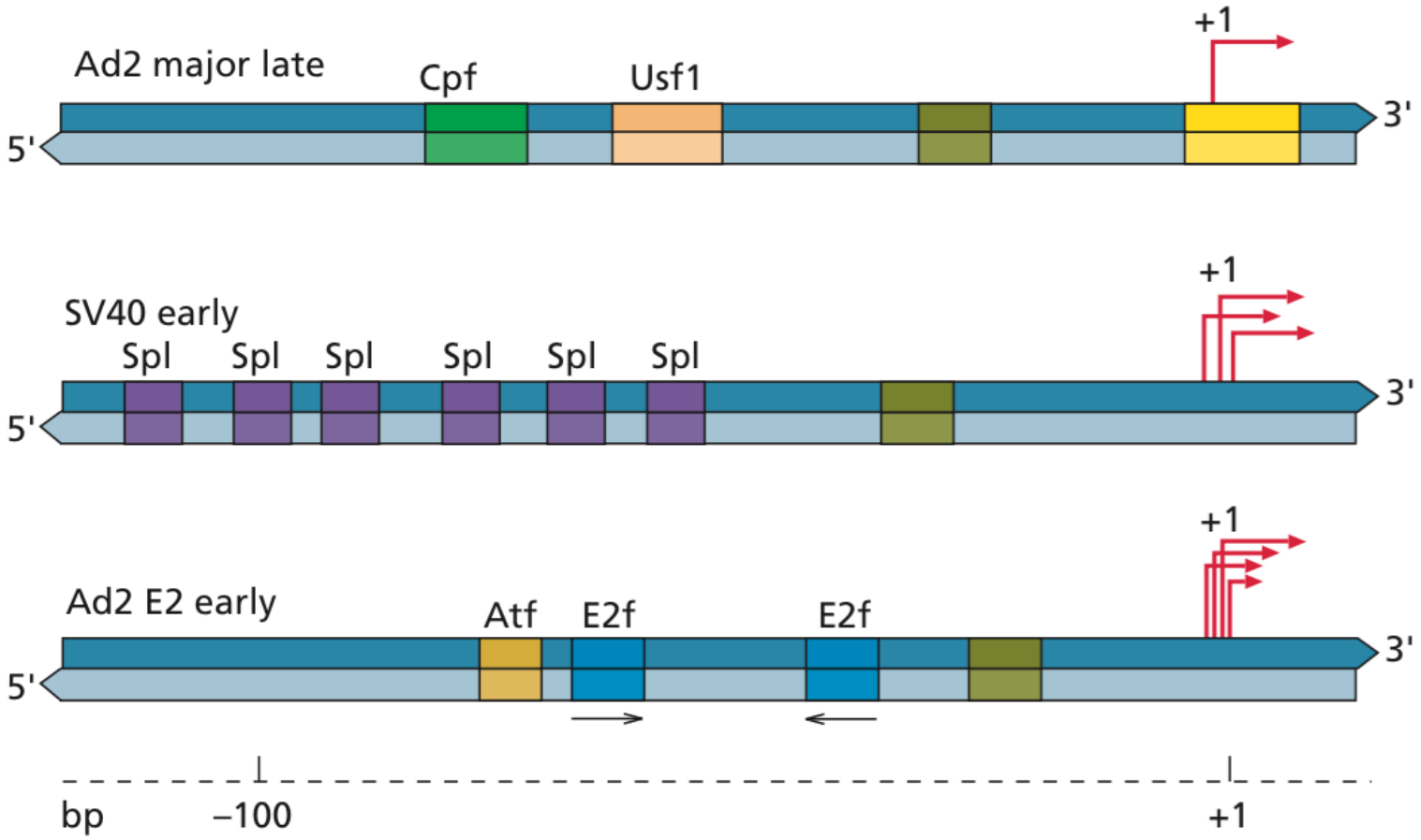


Transcription is regulated

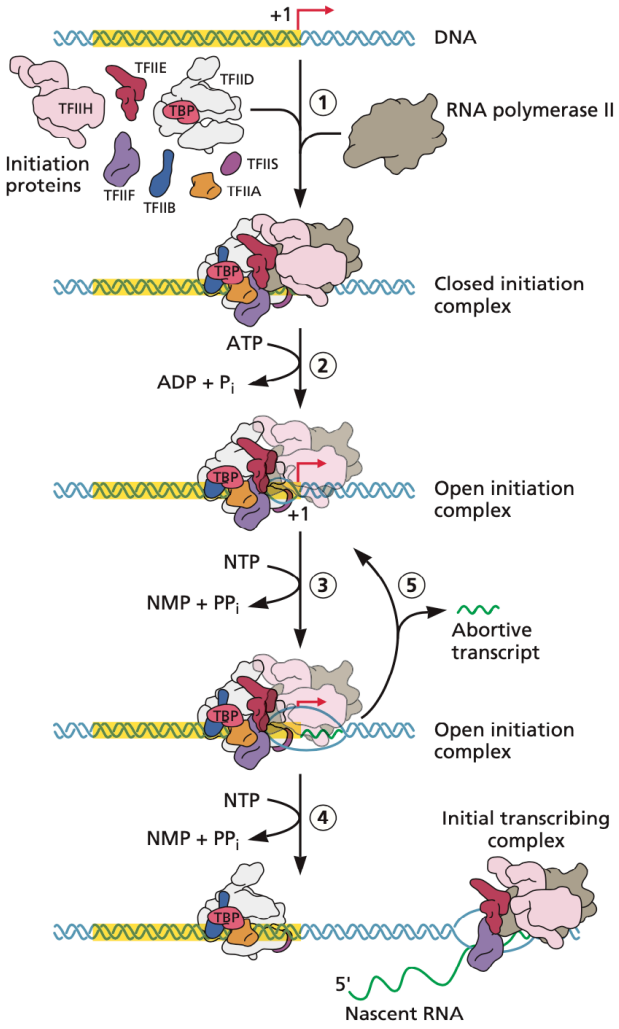


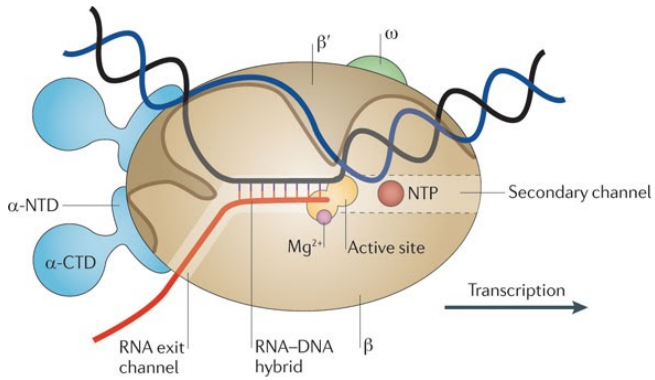
(Specific DNA sequences that bind proteins)

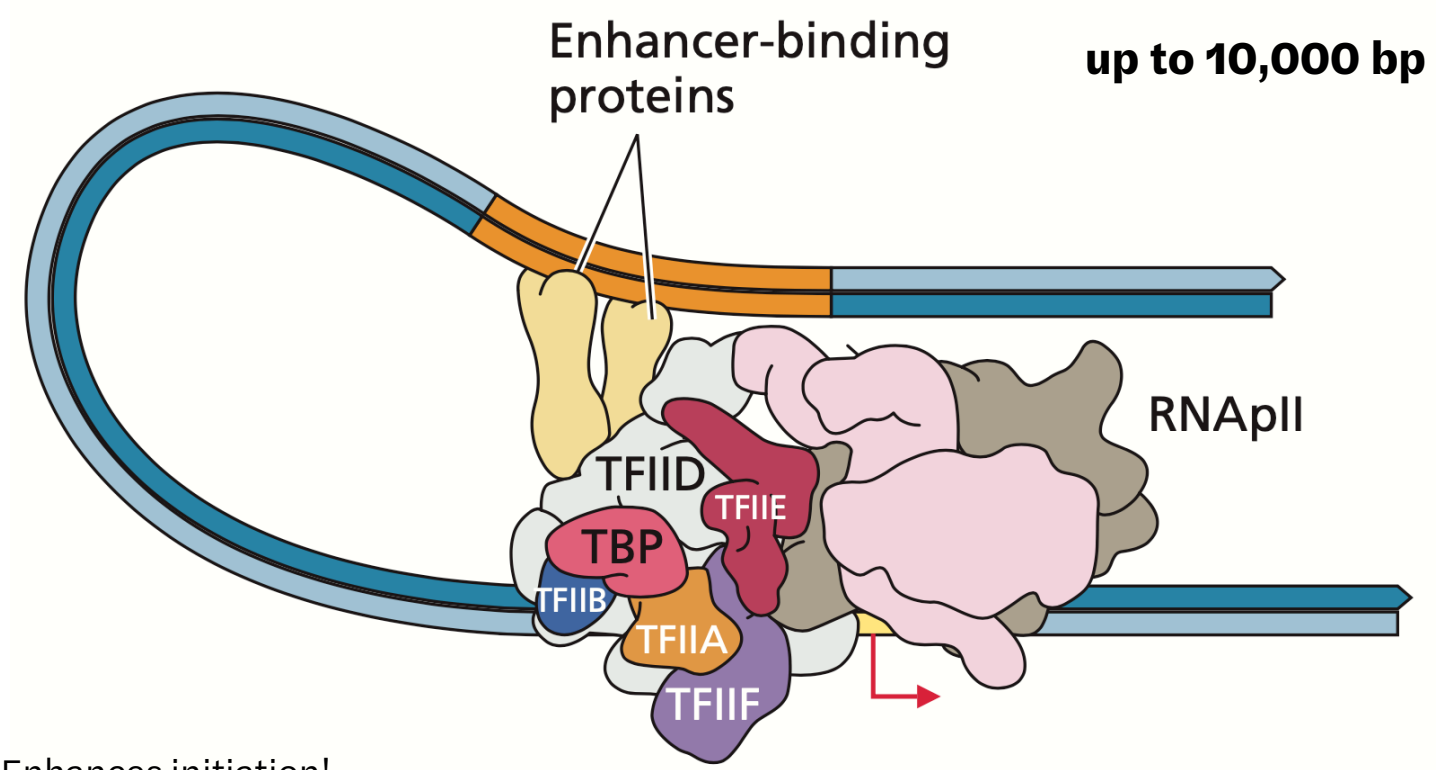
Regulatory sequences in transcriptional control regions



Initiation of transcription by RNA polII





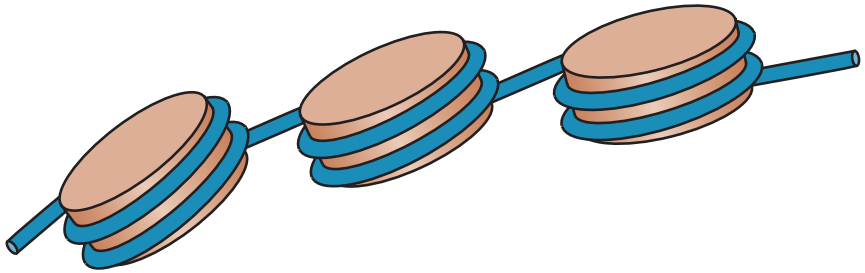


Enhances initiation!

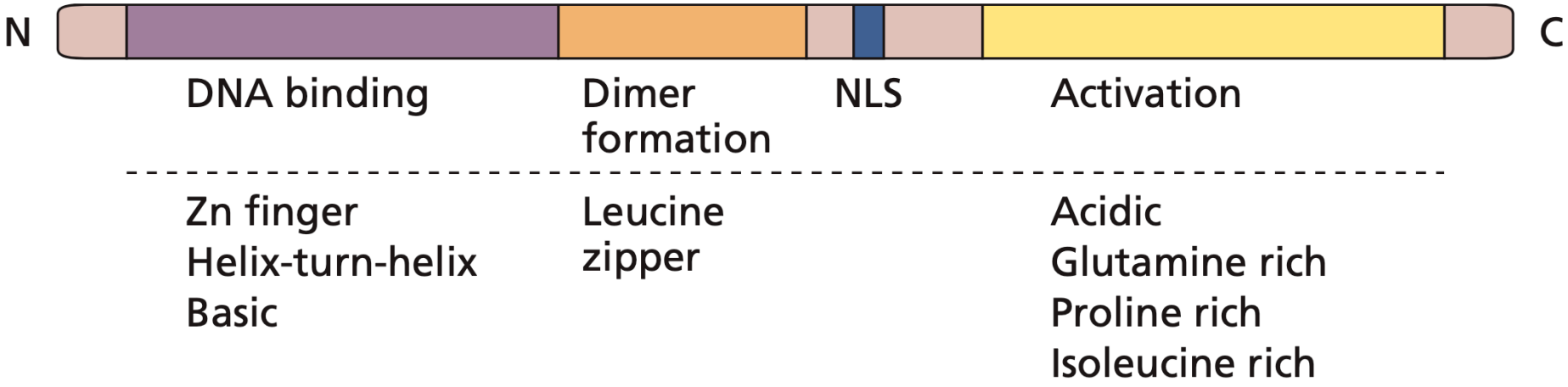
Initiation complex

Proteins that regulate transcription

- Host and/or virus sequence-specific DNA binding proteins
- Viral co-activating molecules (do not bind DNA but can modulate transcription) also required
- Many co-activators modulate structure/activity of nucleosomal templates (i.e. histone methylation or acetylation)



Modular organization of sequence-specific transcriptional activators



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What is the first biosynthetic event that occurs in cells infected with dsDNA viruses?

A. Membrane fusion
B. Transcription
C. DNA replication
D. Protein synthesis
E. All of the above

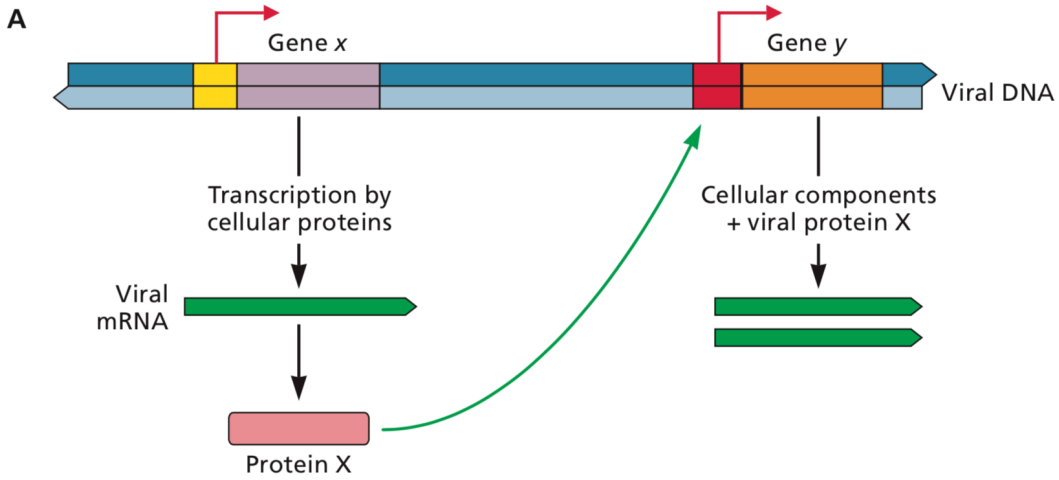
Strategies of transcription of viral DNA

Origin of transcriptional components	Virus
Host only	Retroviruses with simple genomes Caulimoviruses Circoviruses
Host plus one viral protein	
Viral protein transcribes late genes	Bacteriophages T3, T7
Viral protein regulates transcription	Hepadnaviruses, parvoviruses, papillomaviruses, polyomaviruses, retroviruses with complex genomes
Host plus >1 viral protein that stimulate transcription	Adenoviruses, herpesviruses
Viral	Mimiviruses, Poxviruses

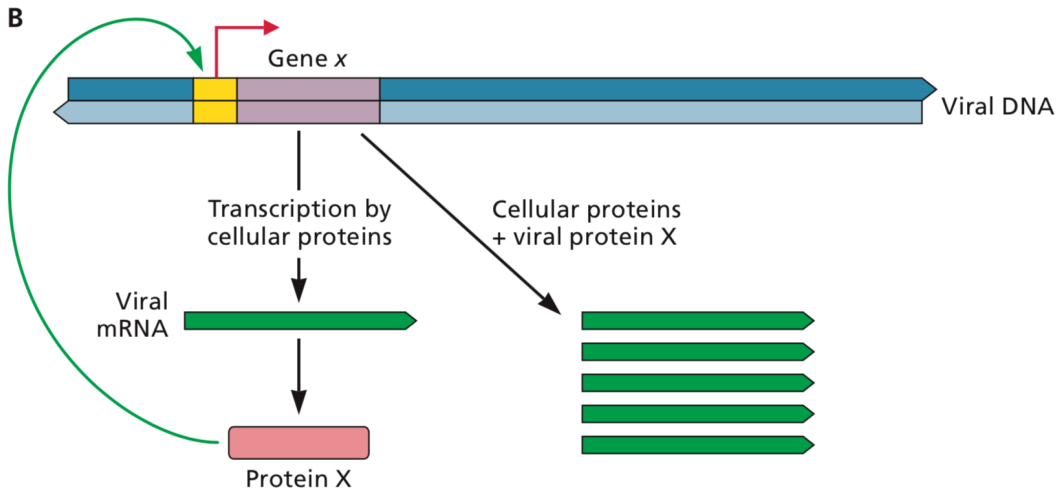
Recognition of viral promoters!

Regulation of transcription by viral proteins

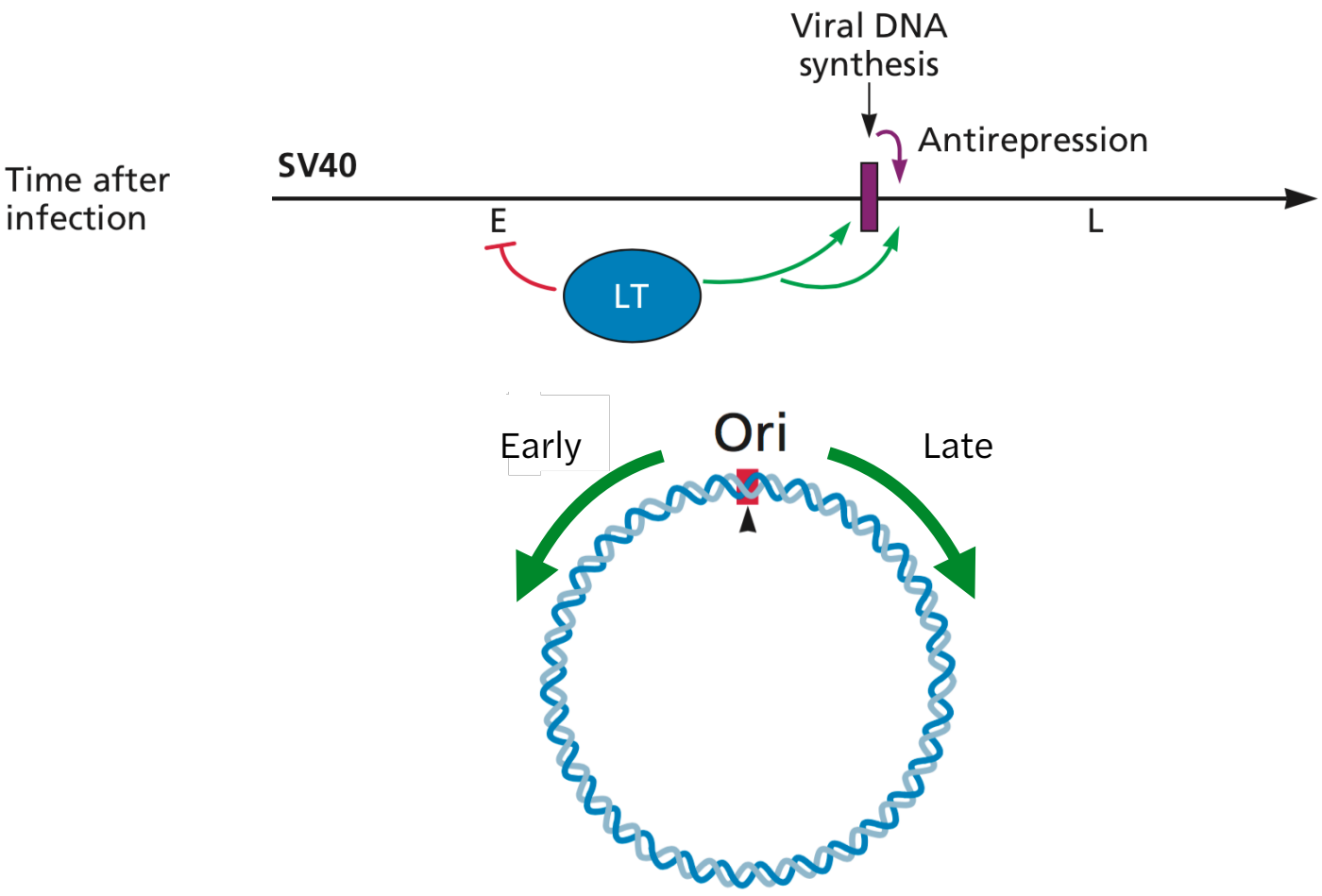
Cascade regulation



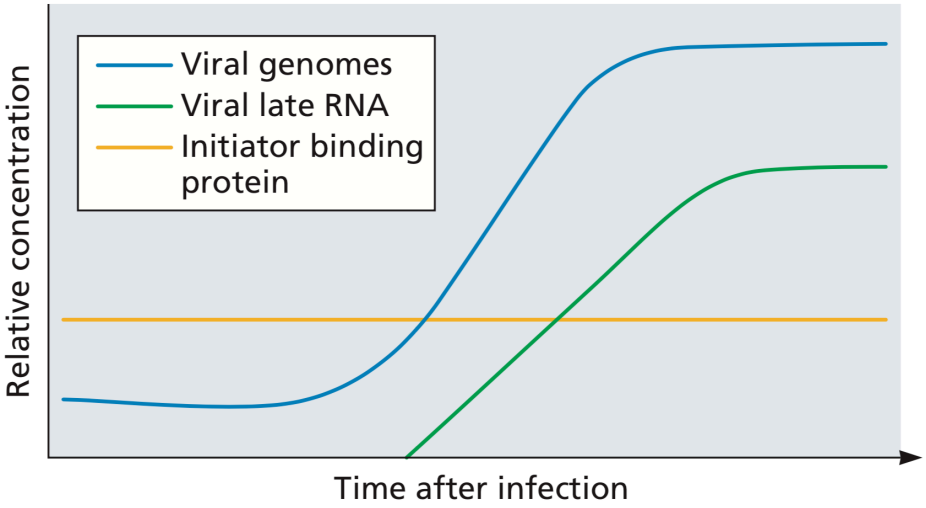
Positive autoregulatory loop (may also be negative)



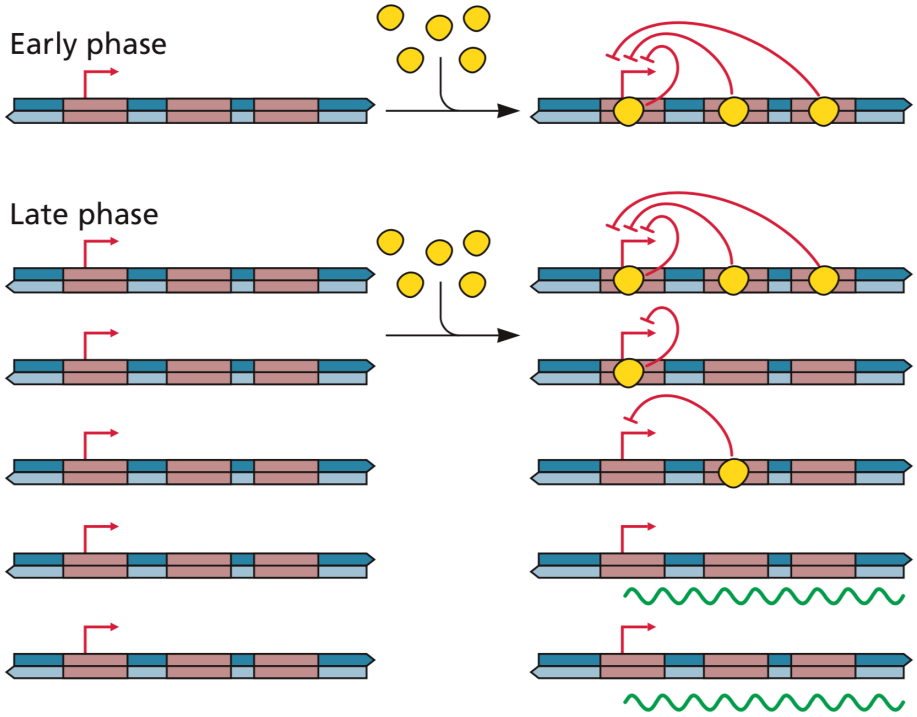
Viral transcriptional programs: SV40

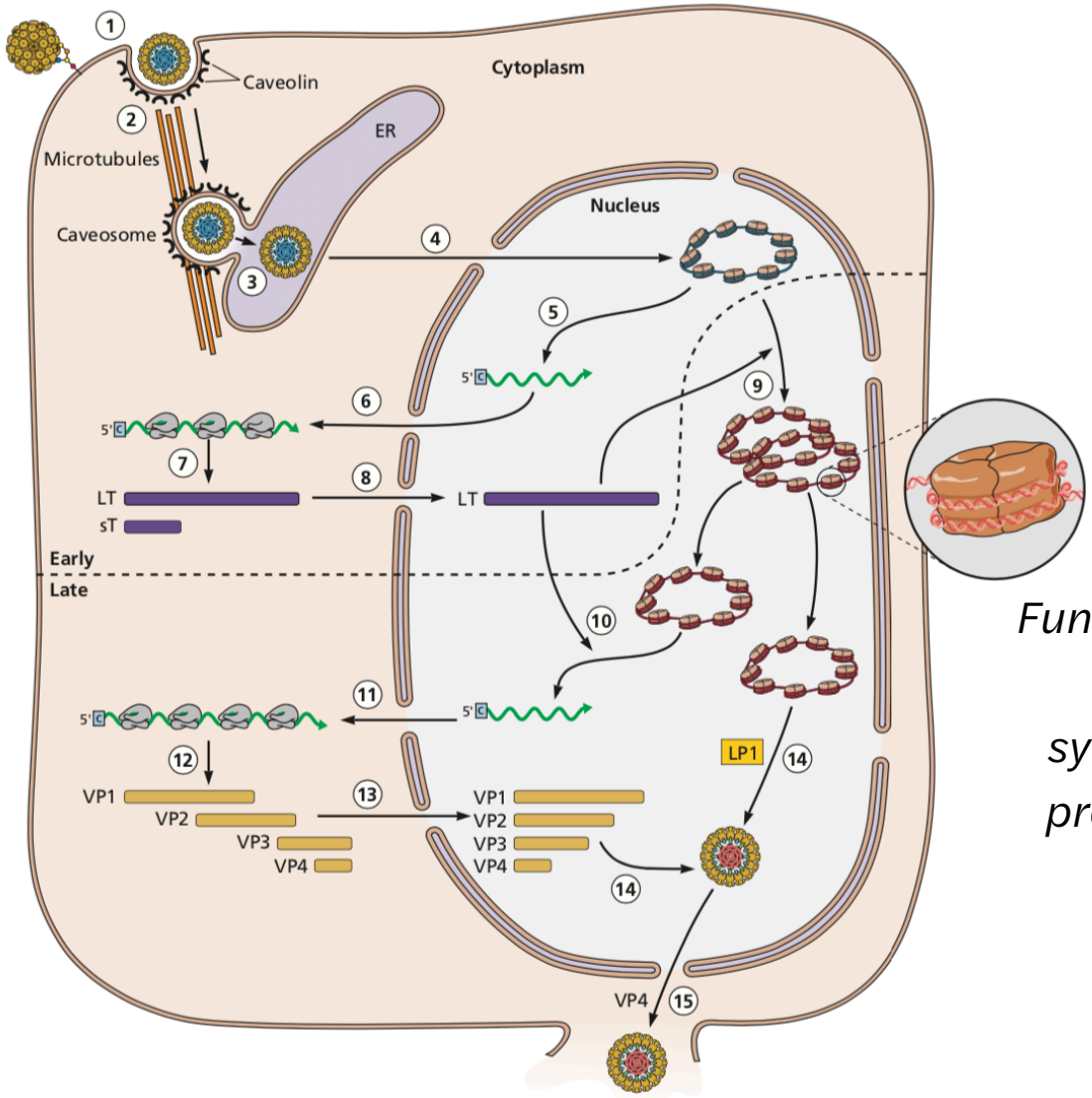


Regulation of SV40 late promoter by cellular repressors



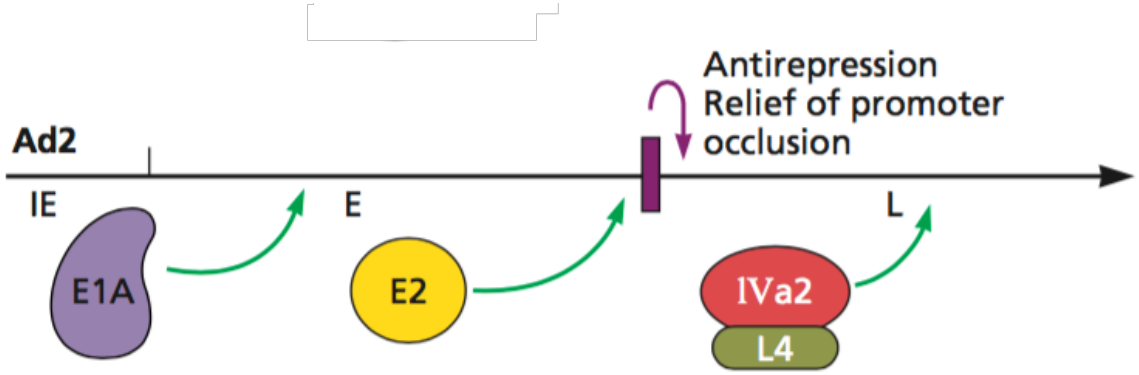
ibp = initiator binding protein, a cell protein





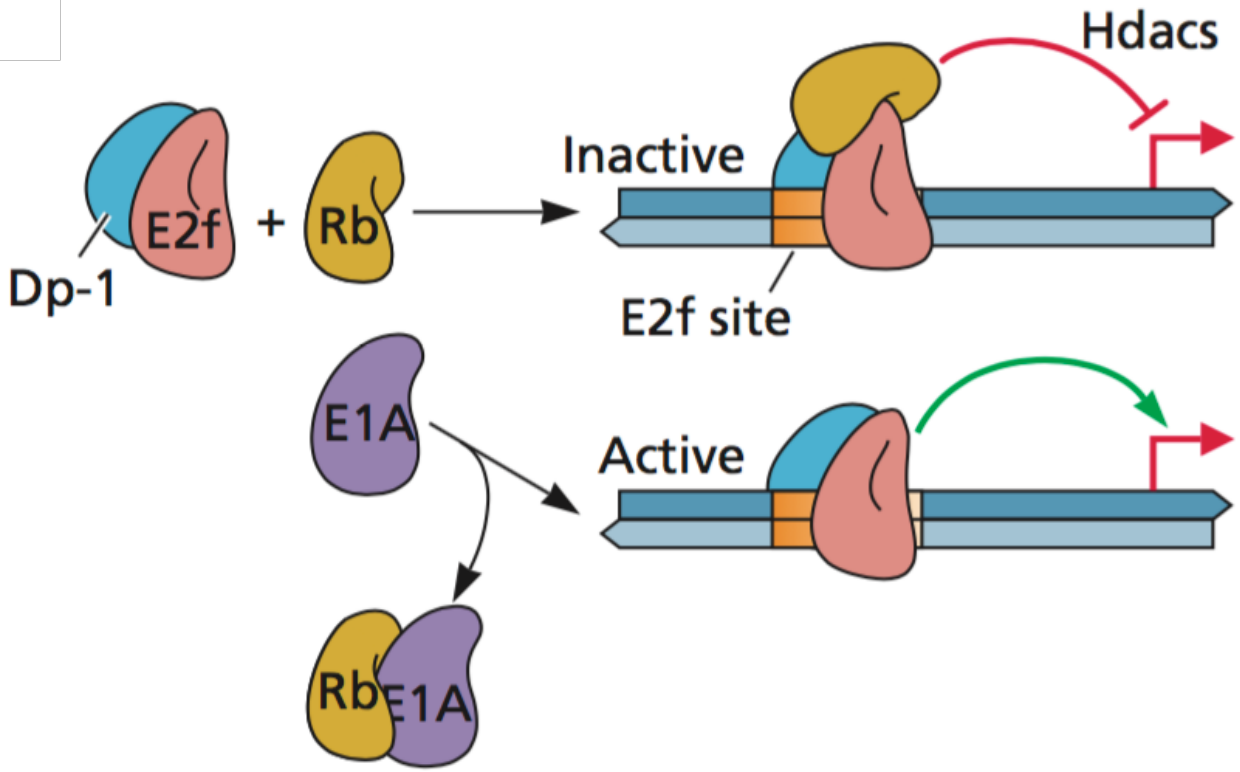
Function of early and late phases? To delay synthesis of structural proteins until DNA has been replicated!

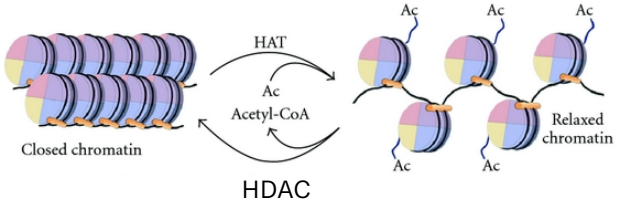
Adenovirus transcriptional regulation



- Three viral proteins and DNA synthesis govern phase transitions
- E1A necessary for transcription of all E transcription units (frees E2f)
- E2 required for DNA synthesis and entry into L phase, increases initiation from major late promoter
- IVa2 enhances L gene transcription

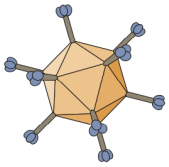
Stimulation of transcription by Ad E1A proteins

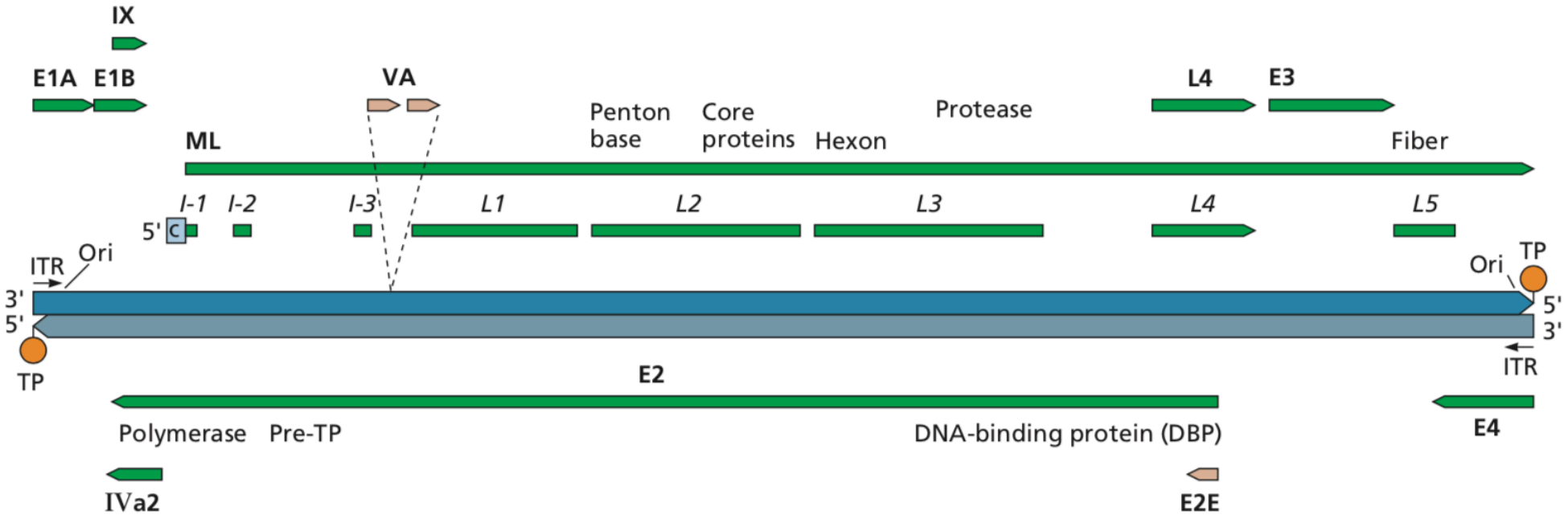


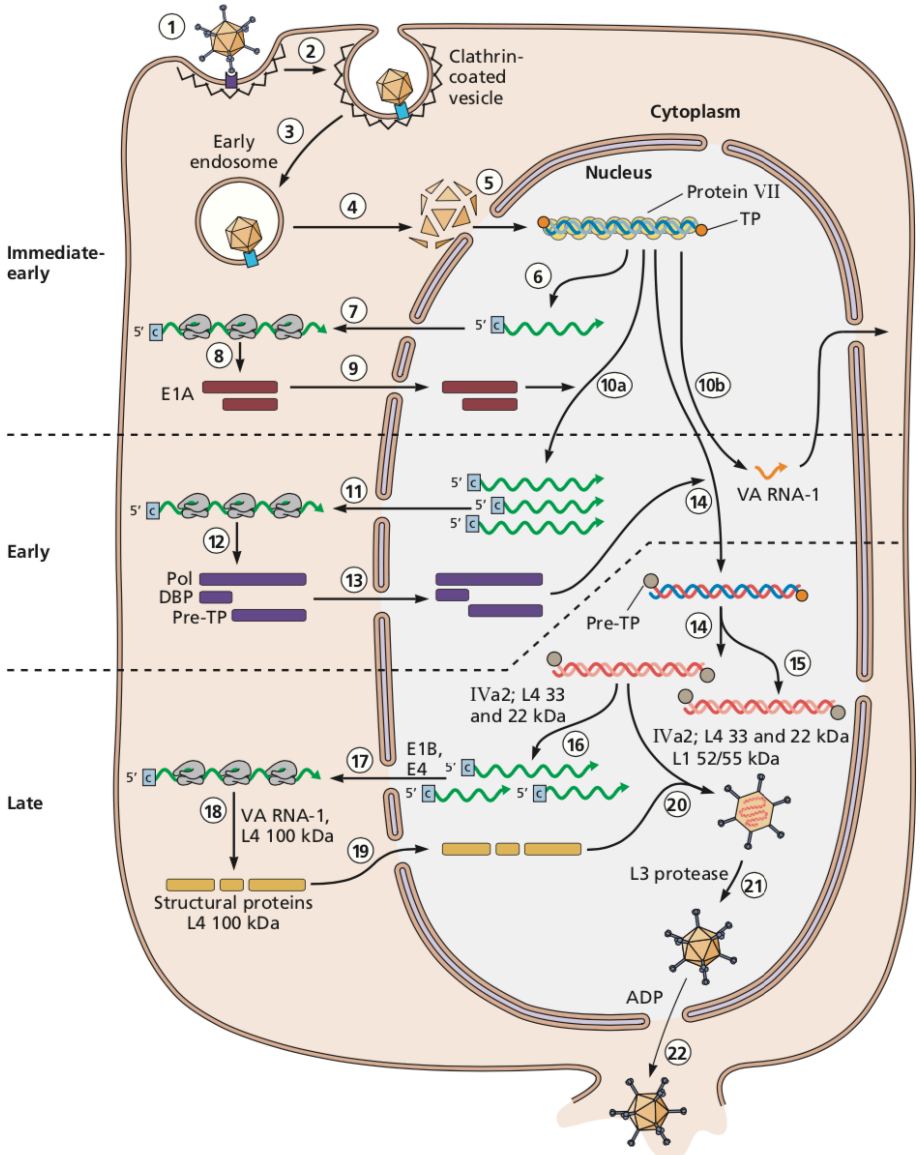


Histone deacetylases remove acetyl groups from histones, wrap DNA more tightly, reducing transcription

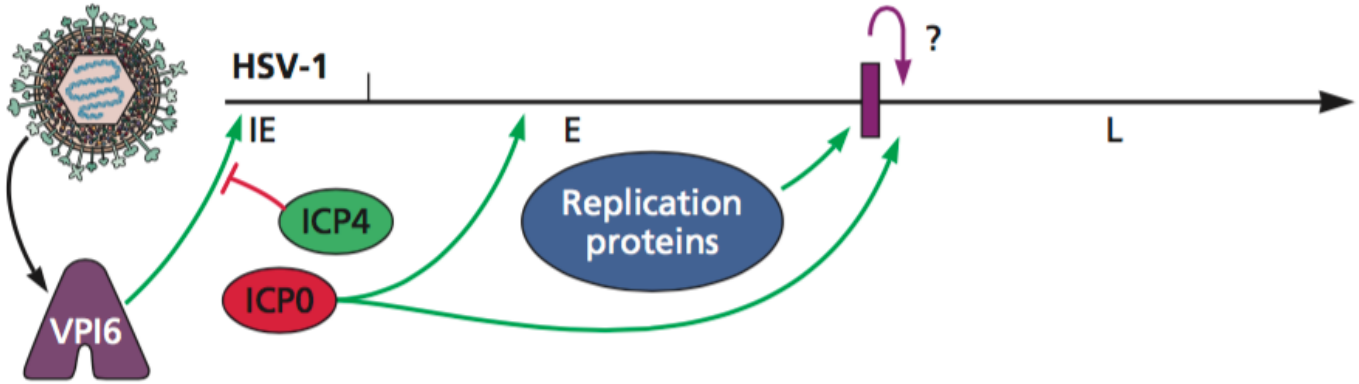
Adenovirus transcription units



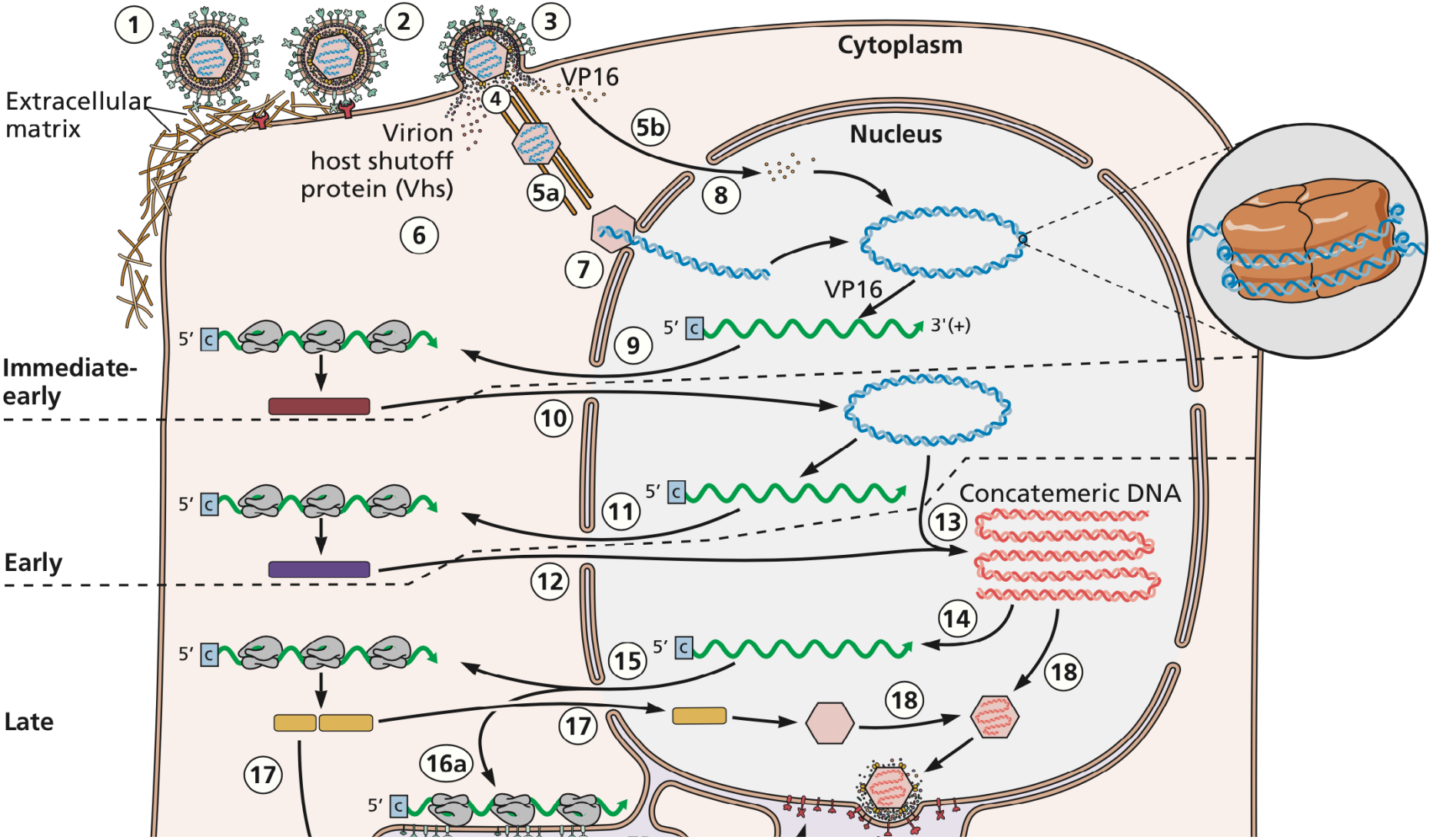




Herpesvirus transcriptional programs



- Initiated by VP16, a virion associated protein (differs from Py, Ad)
- Activates IE transcription - promoter poorly recognized by cell transcription machinery
- IE proteins control transcription from all virus genes
- Expression of E genes and DNA synthesis
- Expression of L genes, DNA dependency
- Ensures coordinated production of DNA genomes and structural proteins



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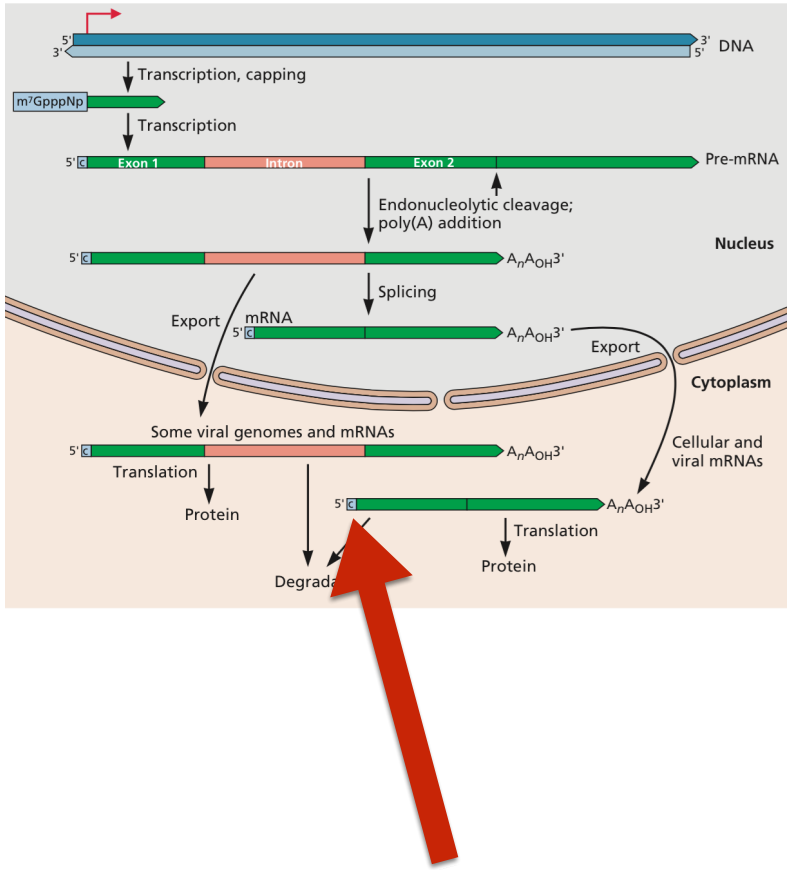
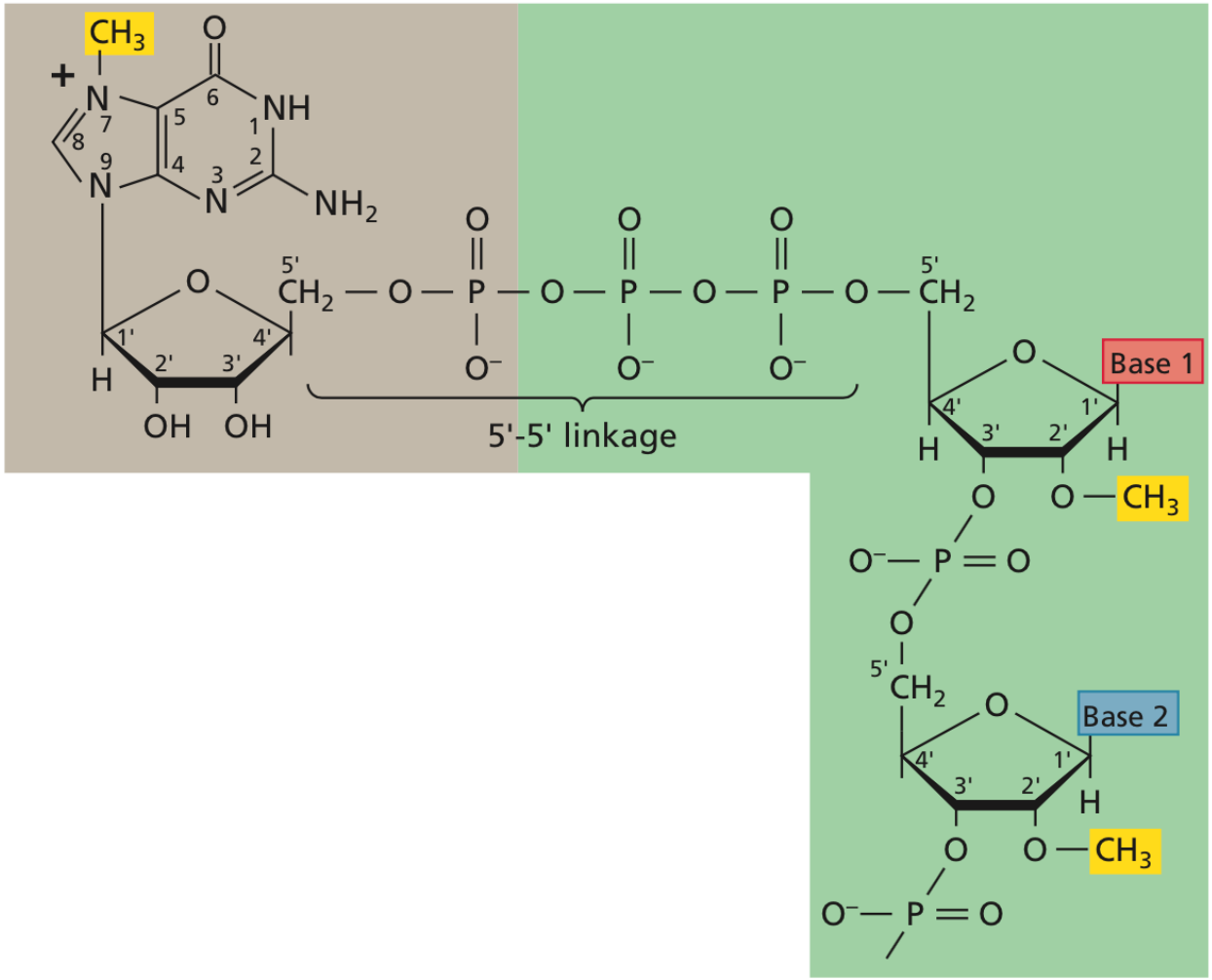
Adenovirus E1A protein stimulating the expression of adenovirus E2 protein which then stimulates the expression of adenovirus IVa2 & L4 protein is an example of:

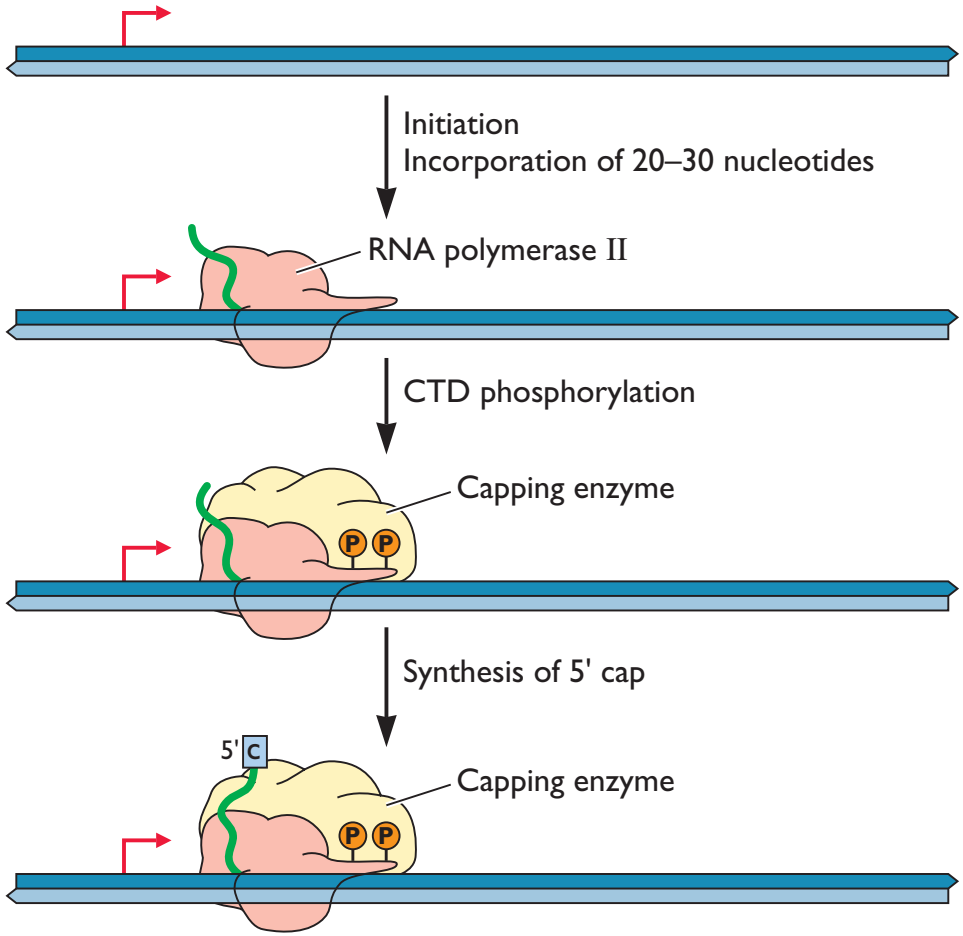
A. A negative autoregulatory loop
B. Repression of gene expression
C. Cascade regulation
D. Dimerization

Modification of mRNA: 5’-cap structure

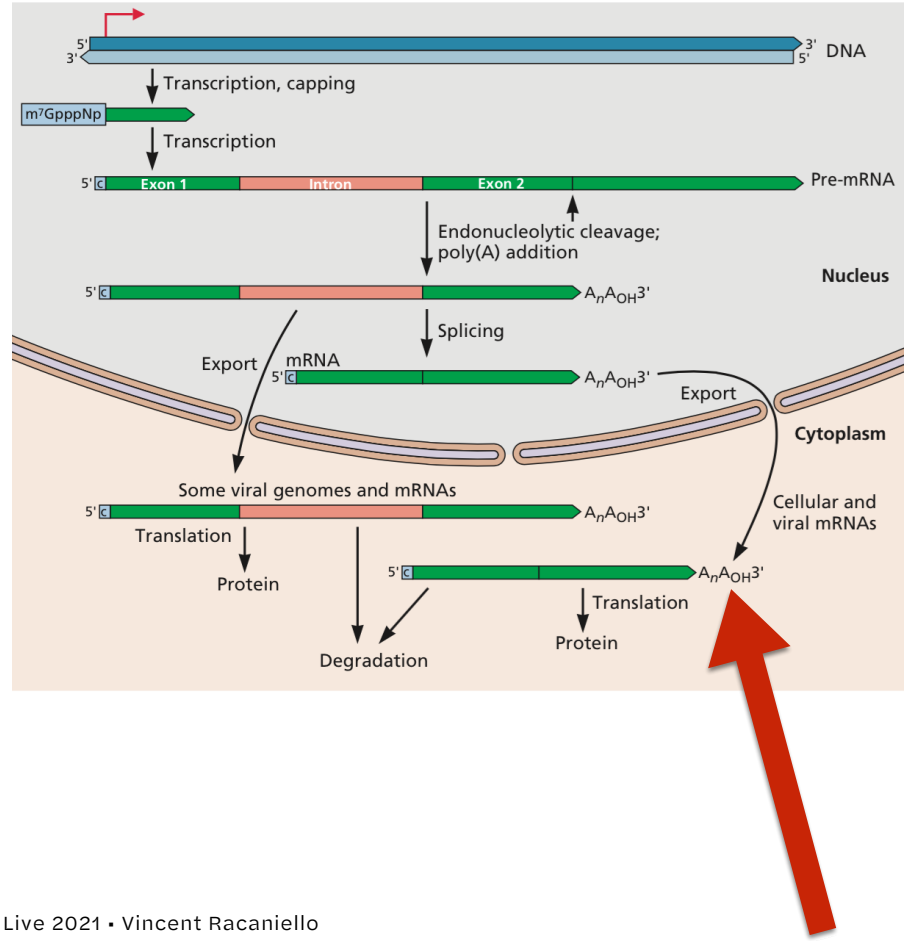


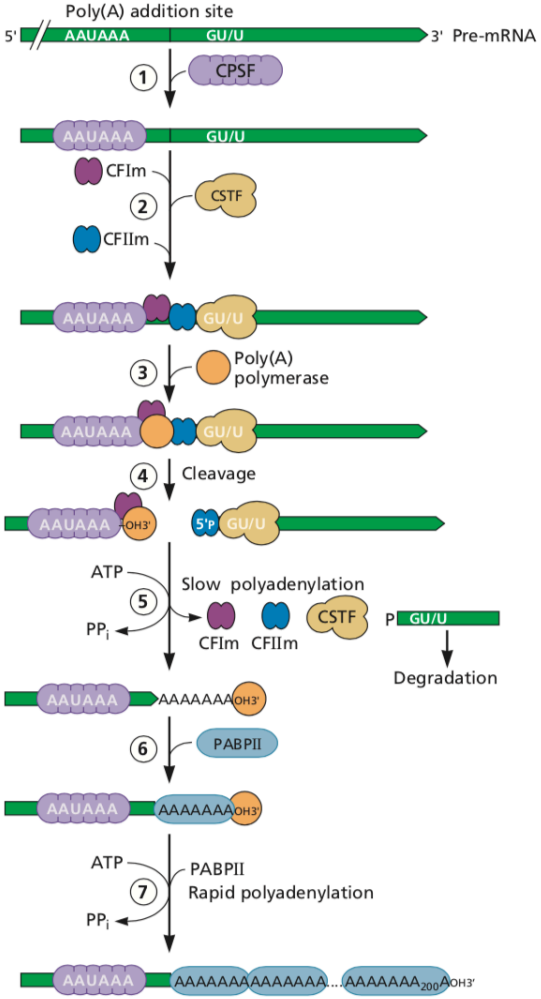


Co-transcriptional capping



Modification of mRNA: Cleavage and polyadenylation



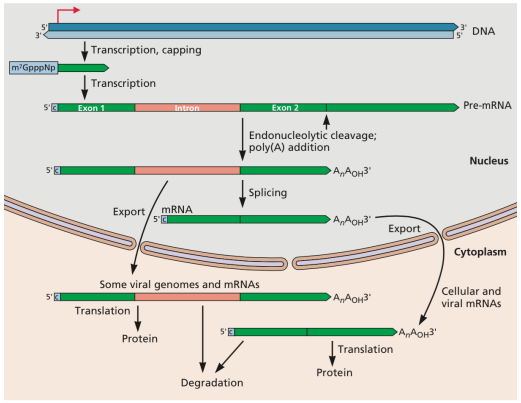


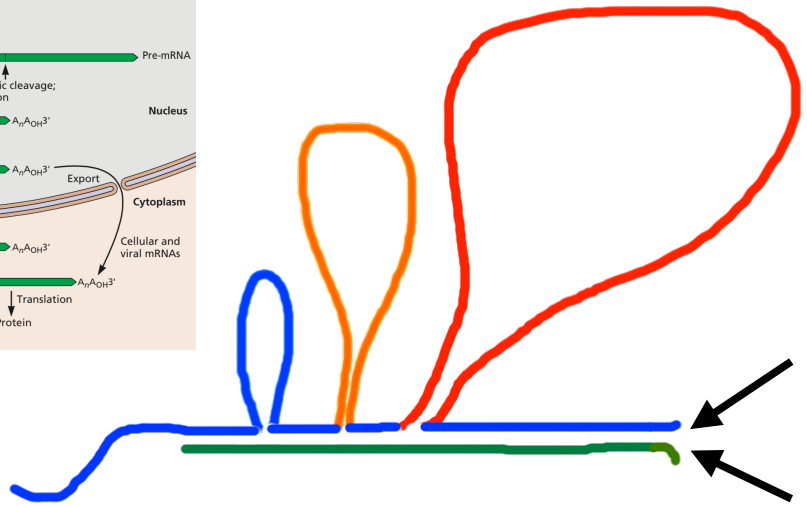
~200 A

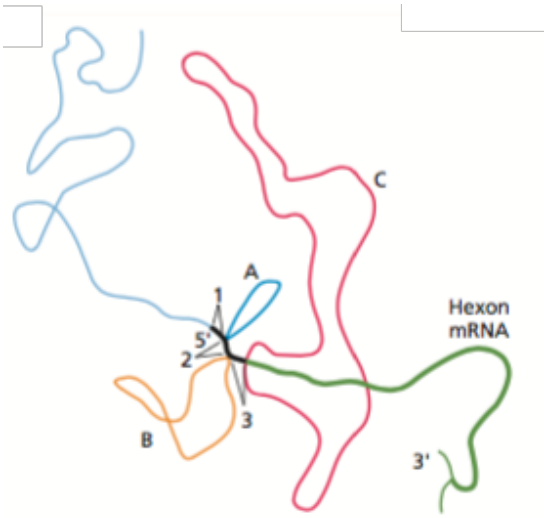
Addition of poly(A) to viral mRNAs

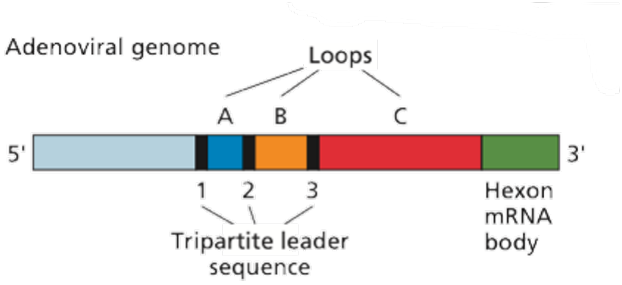
Mechanism	Enzyme	Viruses
Post-transcriptional		
Cleavage of pre-mRNA followed by polyadenylation	Cellular	Adenovirus, HBV, HDV, herpesviruses, polyomavirus, retrovirus
During mRNA synthesis		
Reiterative copying at stretches of U in template RNA	Viral	Influenza virus, VSV
Copying of long U stretch in template RNA	Viral	Poliovirus, alphavirus

Discovery of mRNA splicing in adenovirus infected cells



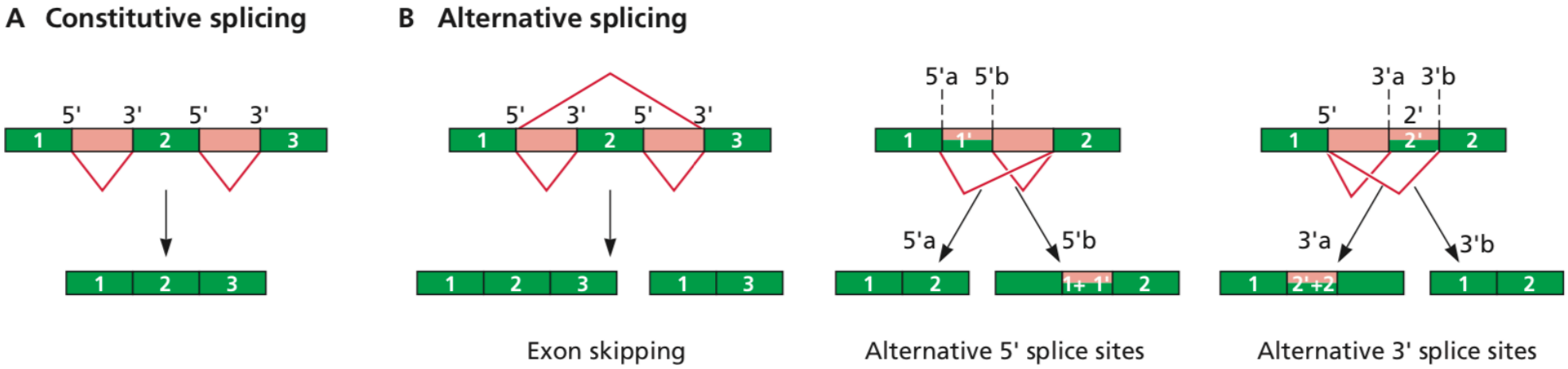


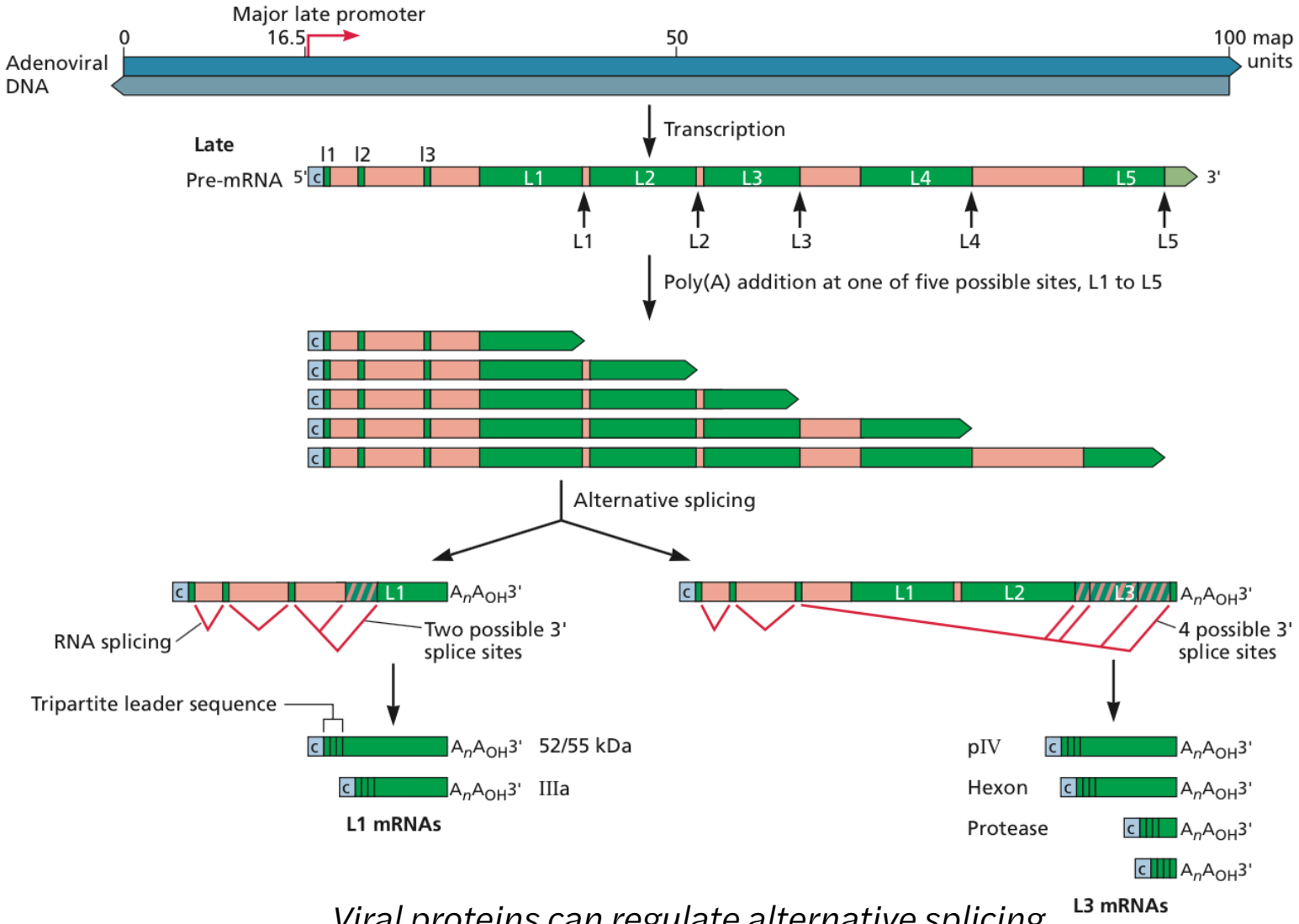




Nobel Prize, Physiology or Medicine, 1993, to Richard J. Roberts and Phillip A. Sharp for their discovery of split genes

Constitutive and alternative splicing





Viral proteins can regulate alternative splicing

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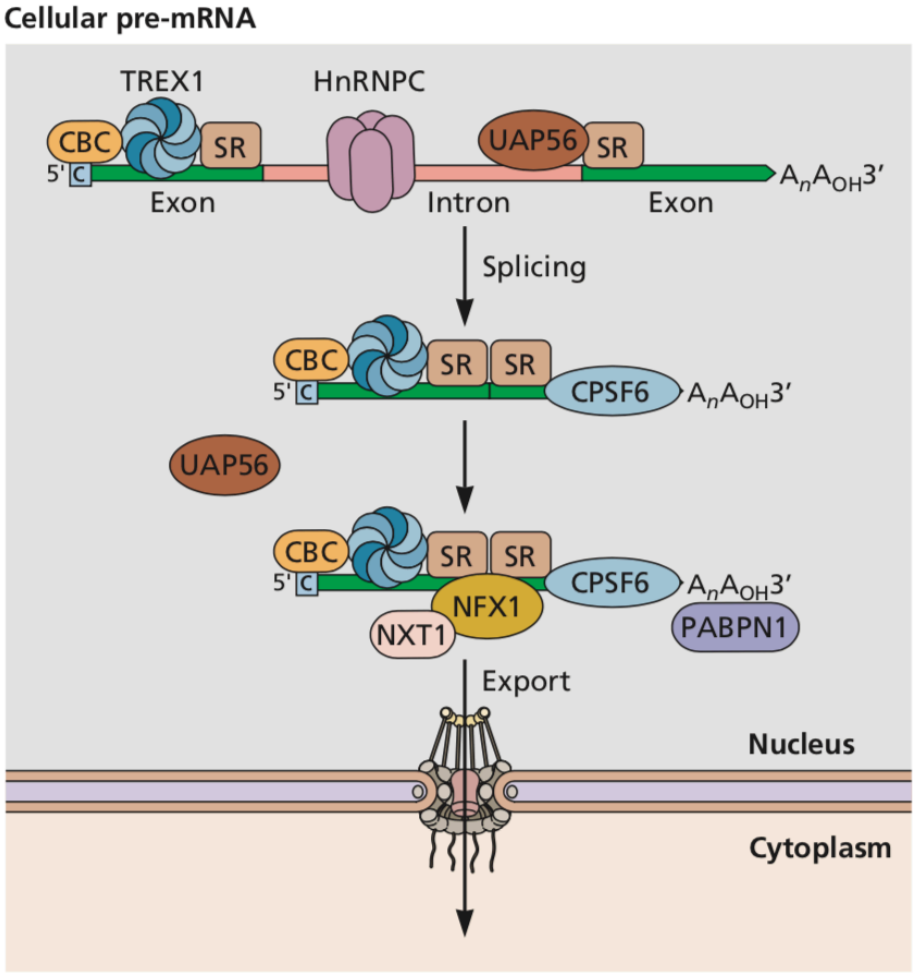
Which statement about polyadenylation of DNA virus mRNAs is correct?

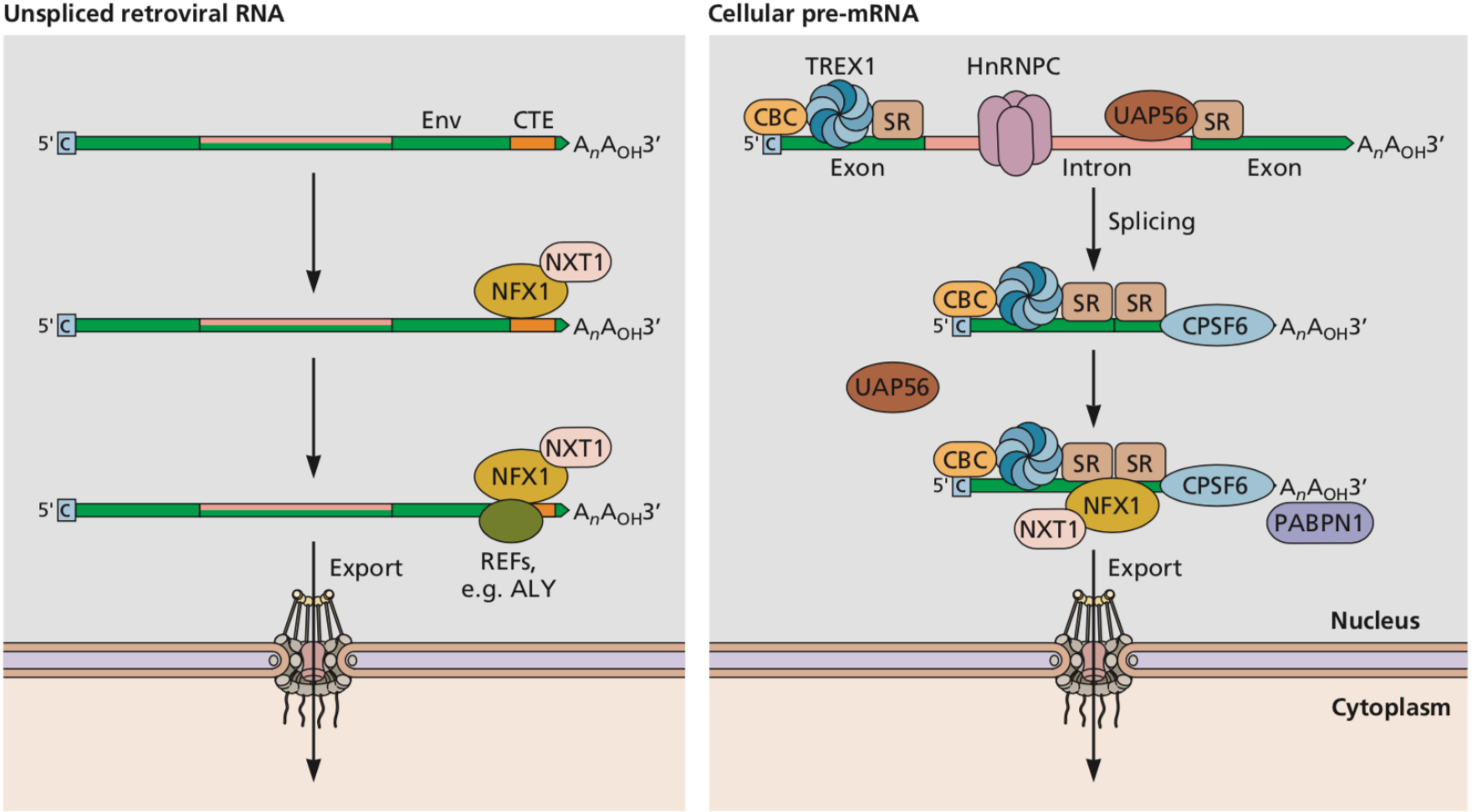
A. It always occurs in the cytoplasm
B. It occurs after cleavage of pre-mRNA
C. Poly(A) is added at the 5'-end of pre-mRNA
D. Is specified by a stretch of U residues in the template

Splicing marks mRNAs for nuclear export



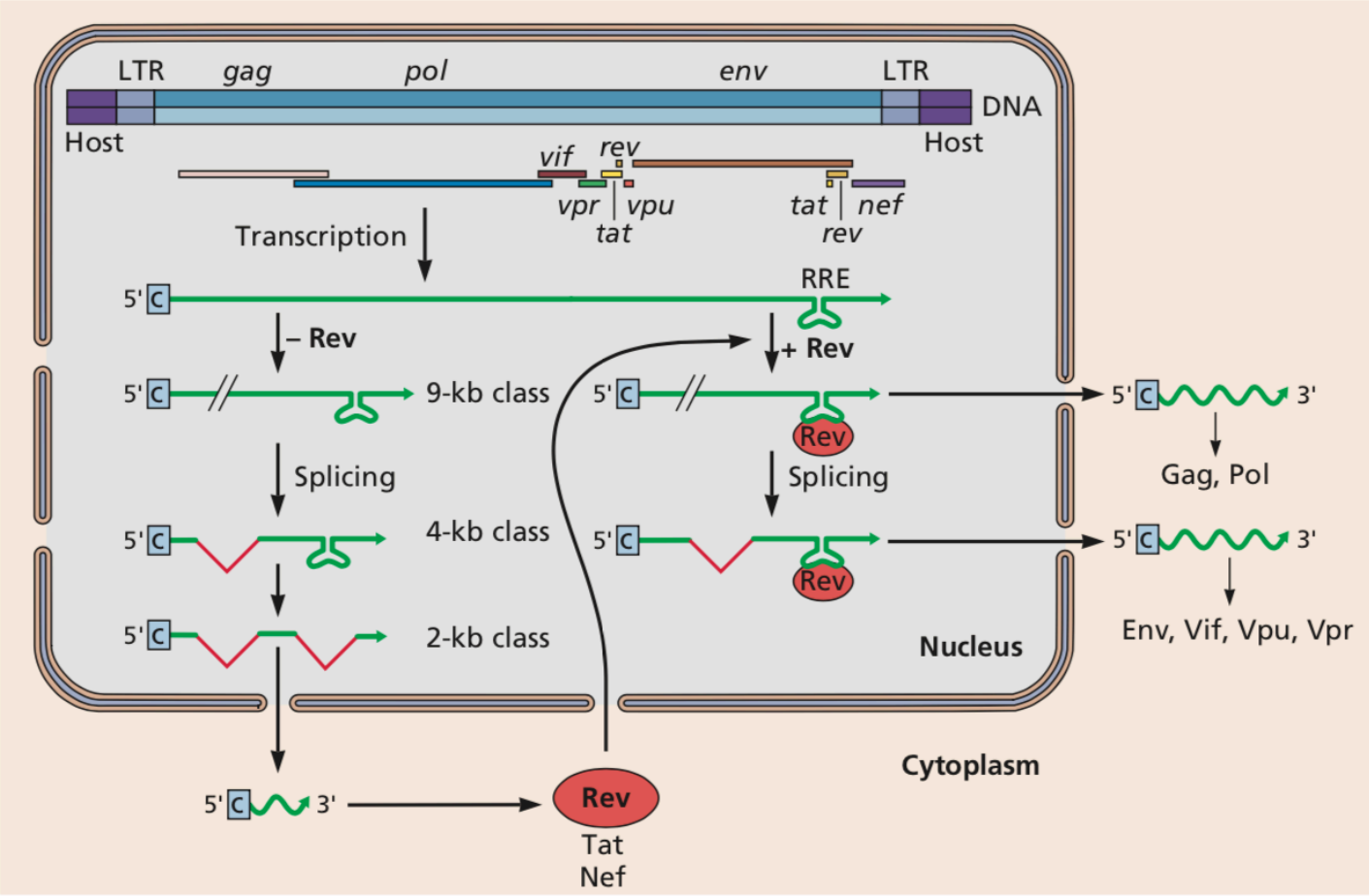
components of nuclear export pathway

Export of unspliced retroviral mRNA

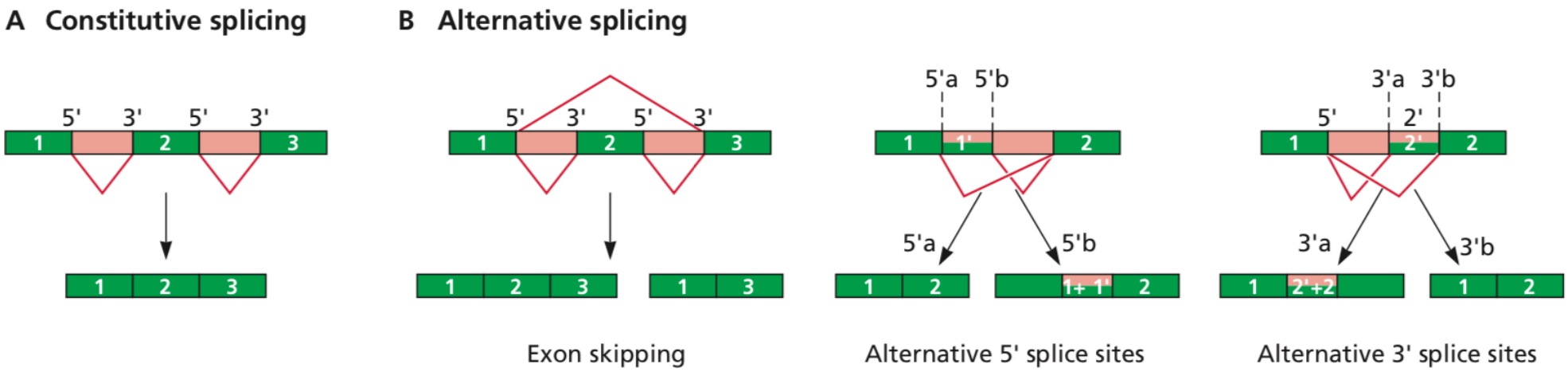


CTE = Constitutive transport element

Rev protein regulates export of HIV mRNA

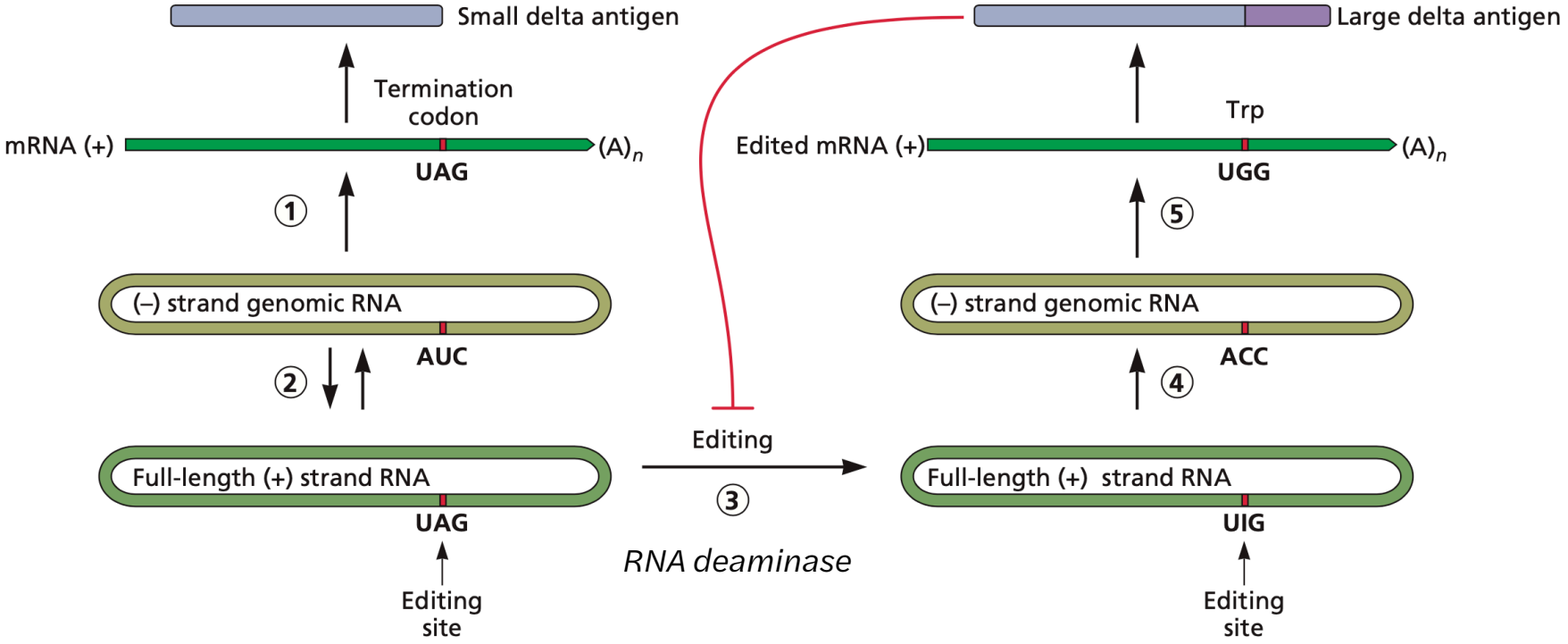


Splicing = Value added



- Alternative splicing creates different mRNAs, proteins
- Coding information of a small DNA genome is expanded
- Regulation of gene expression

RNA editing


Small delta antigen
Large delta antigen
Termination codon
Trp
mRNA (+)
(A)n
UAG
Edited mRNA (+)
(A)n
UGG
1
5
(–) strand genomic RNA
AUC
(–) strand genomic RNA
ACC
2
4
Editing
Full-length (+) strand RNA
UAG
3
Full-length (+) strand RNA
UIG
RNA deaminase
Editing site
Editing site

Noncoding RNAs

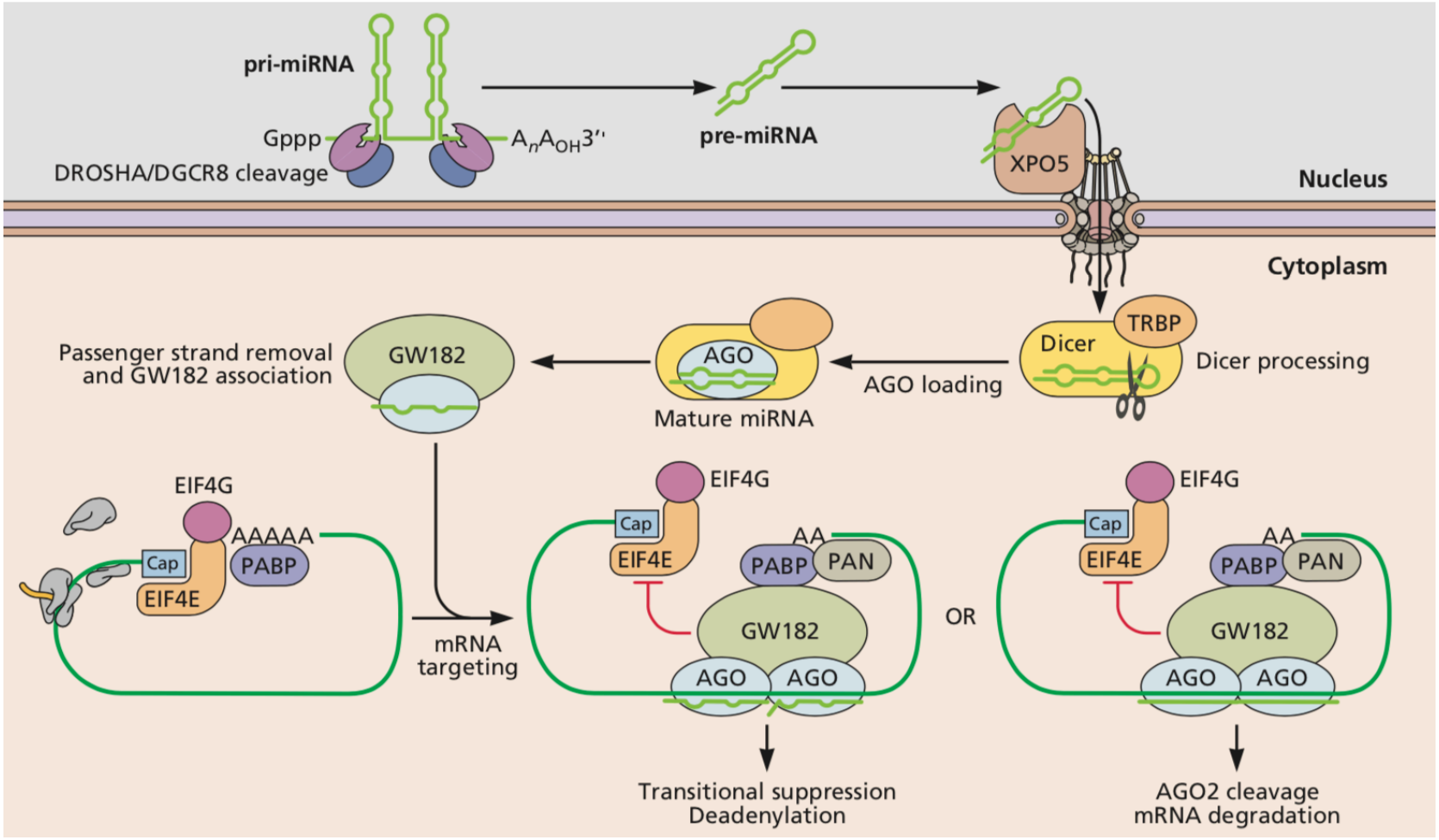
- Besides tRNAs and rRNAs, eukaryotic cells contain a large repertoire of noncoding RNAs
- Most human transcripts do not encode proteins
- Classified into short (<200 bases) and long (>200 bases)
- Perform a variety of regulatory functions
- Viral genomes contain noncoding RNAs

miRNAs

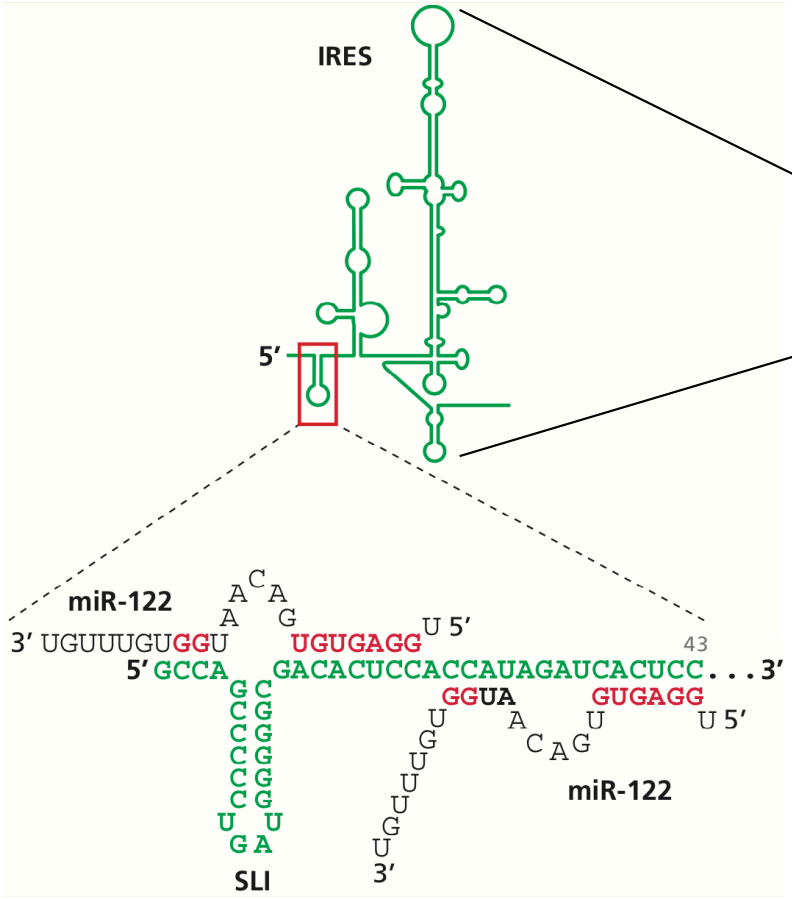
lncRNAs

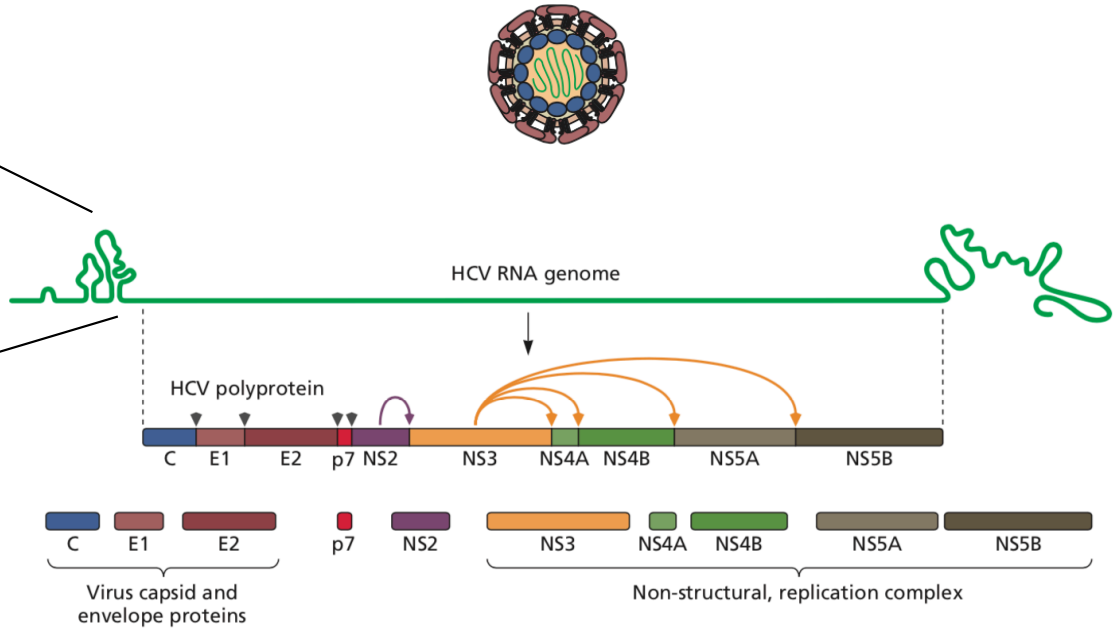
circRNAs

Synthesis and function of miRNAs

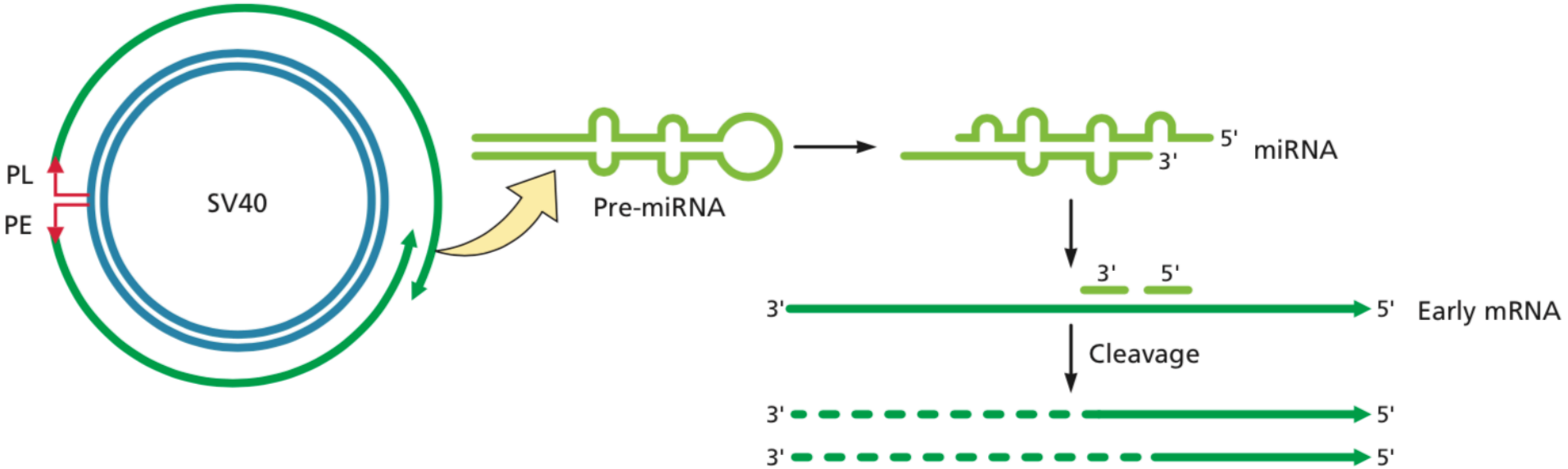


Liver-specific miR-122 promotes hepatitis C virus replication



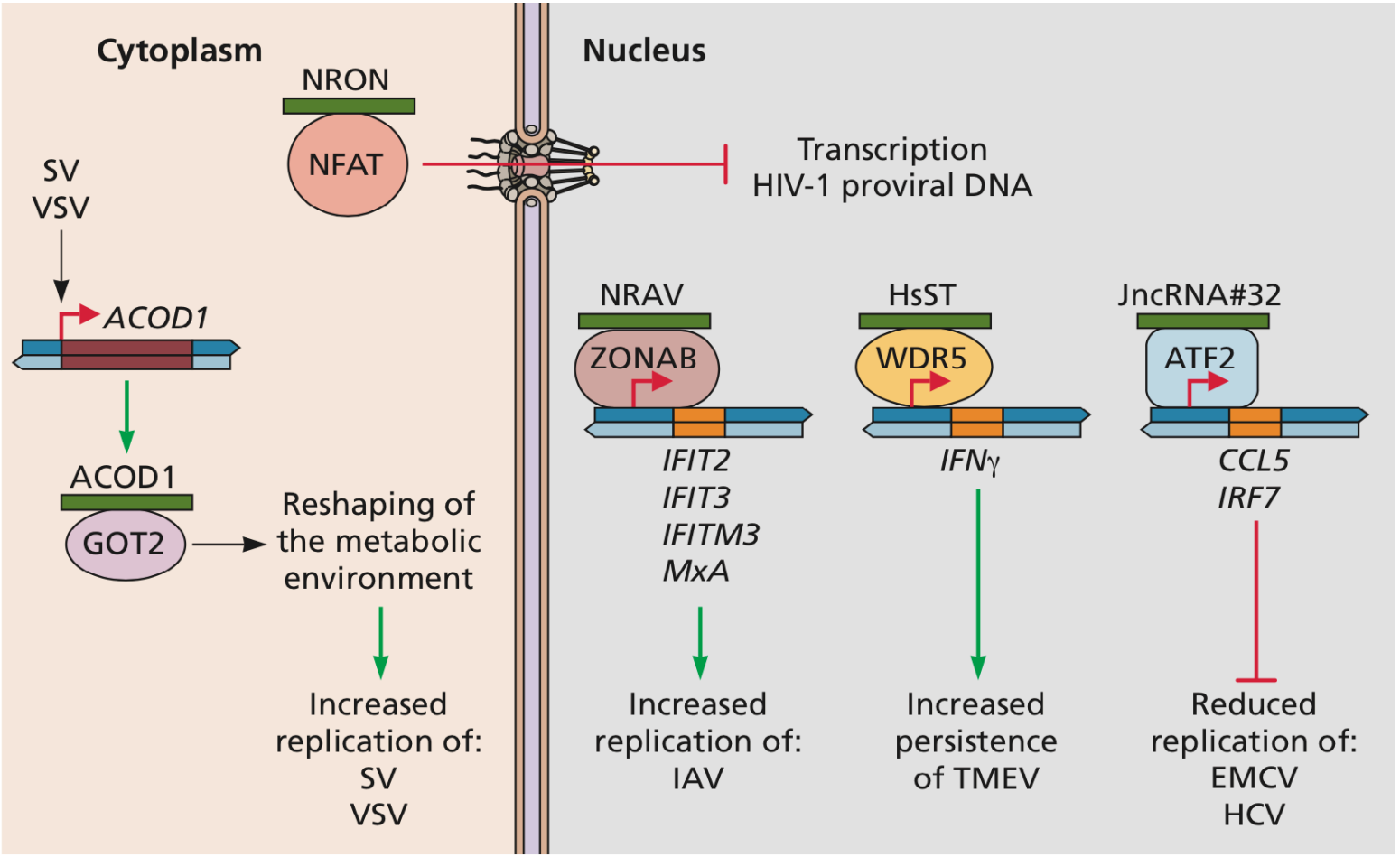


Polyomavirus miRNA may promote persistence

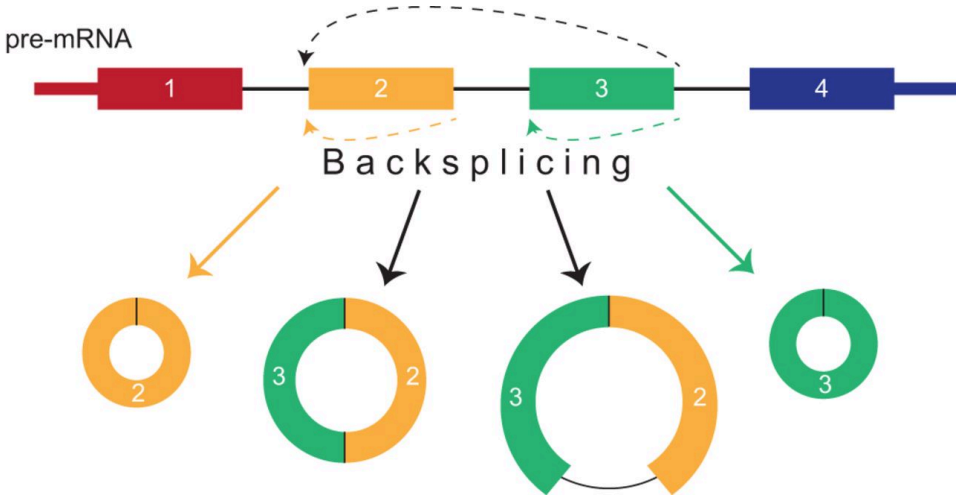


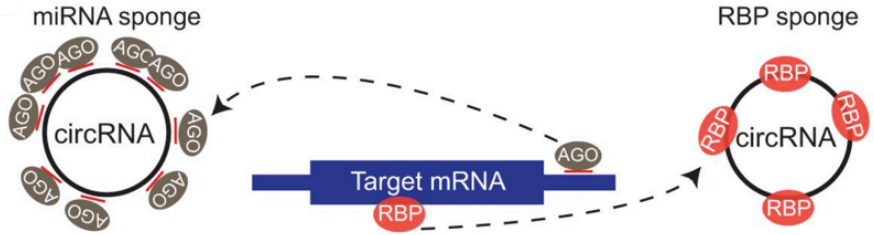
Both miRNAs are perfectly complementary to specific sequences in the early mRNAs that encode LT and induce its cleavage.

Effect of lncRNAs on virus reproduction



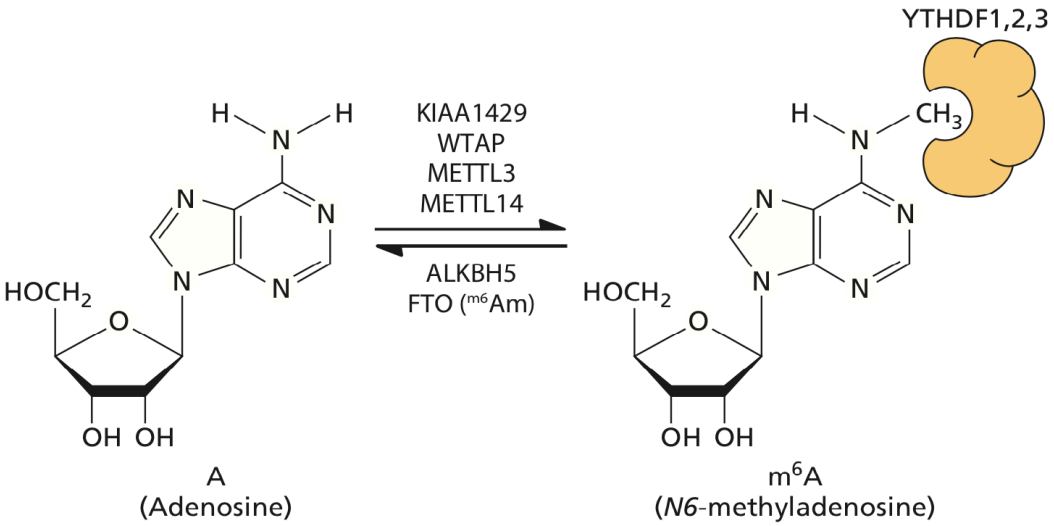
Circular RNAs (circRNAs) produced by back-splicing

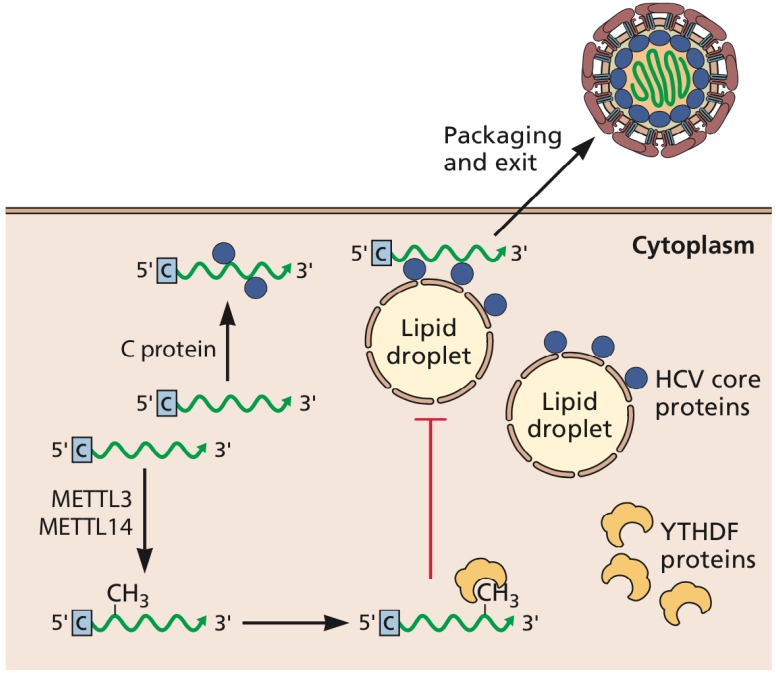




Highly abundant in uninfected and some virus-infected cells

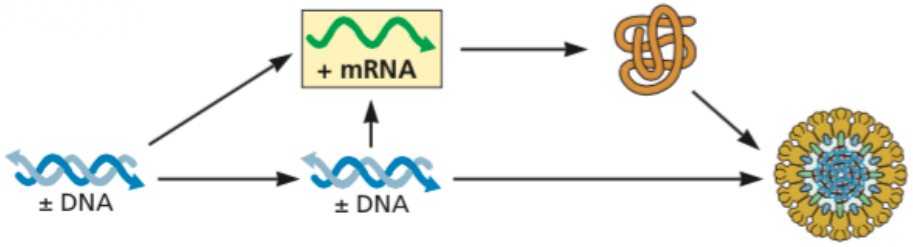
Reversible N6 methylation of internal adenosine nucleosides



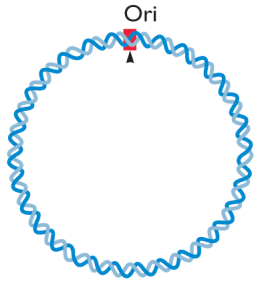


N6A writers, readers, and erasers

DNA genomes

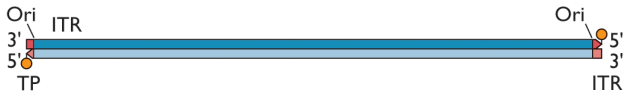


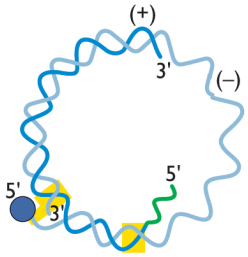
***Polyomaviridae* (5 kbp)**



***Circoviridae* (1.7–2.2 kb)**

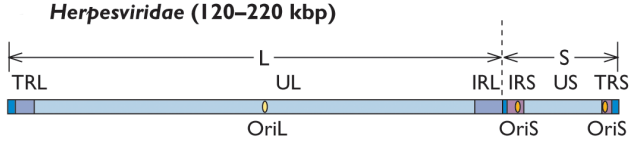
***Adenoviridae* (36–48 kbp)**





***Parvoviridae* (4–6 kb)**

***Herpesviridae* (120–220 kbp)**



***Poxviridae* (130–375 kbp)**

Next time: Viral DNA replication