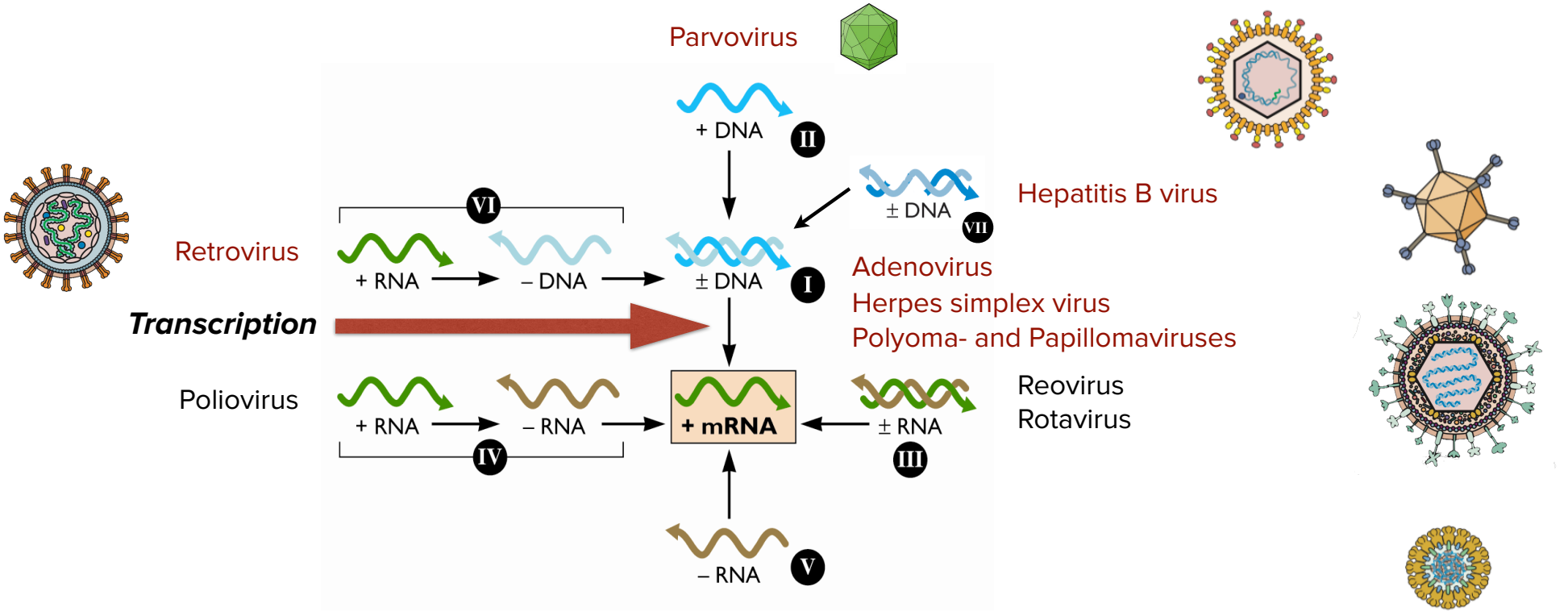
Transcription and RNA processing

Lecture 7
Biology 3310/4310
Virology
Spring 2017

It is possible that Nature invented DNA for the purpose of achieving regulation at the transcriptional rather than at the translational level
—Alan Campbell



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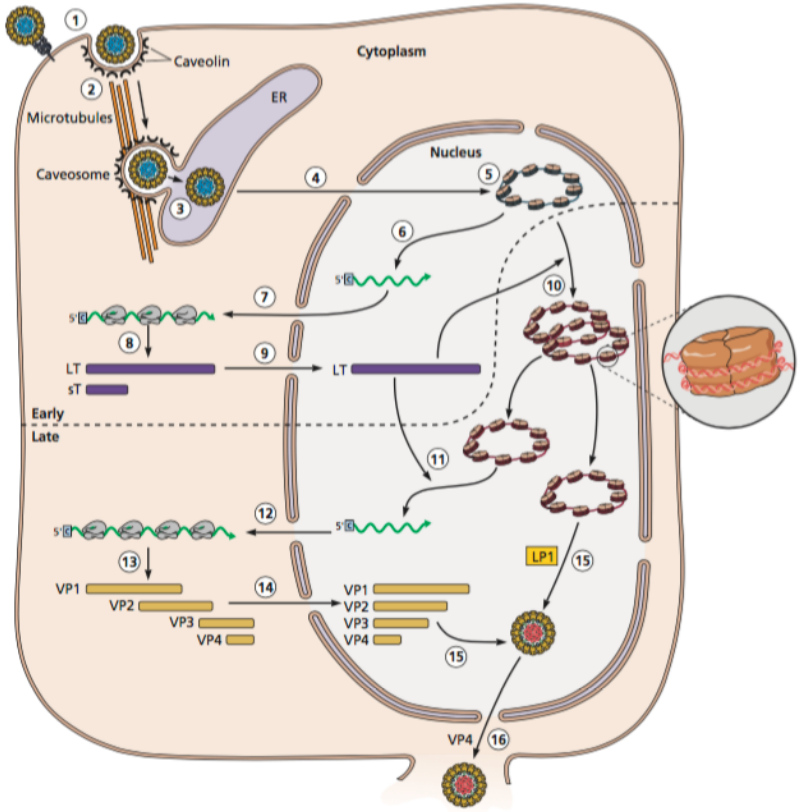
Eukaryotic DNA-dependent RNA polymerases

Enzyme	Cellular RNA	Viral RNA
RNA pol I	Pre-rRNA	None known
RNA pol II	Pre-mRNA Pri-miRNA SnRNA	Pre-mRNA Pri-miRNA HDV genome RNA and mRNA
RNA pol III	Pre-tRNAs 5S rRNA U6 snRNA	Ad-2 VA RNAs EBV EBER RNAs MHV68 pri-miRNA

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

Transcription is the first *biosynthetic* reaction to occur in cells infected with dsDNA viruses



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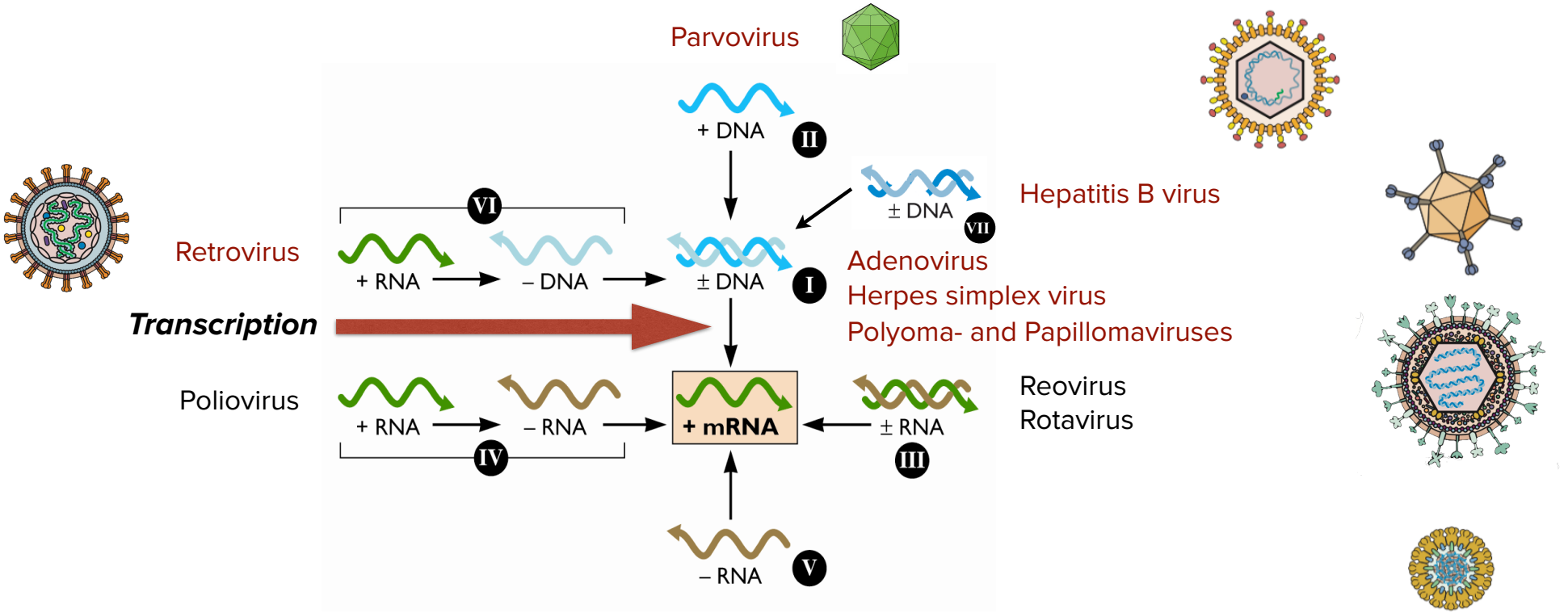

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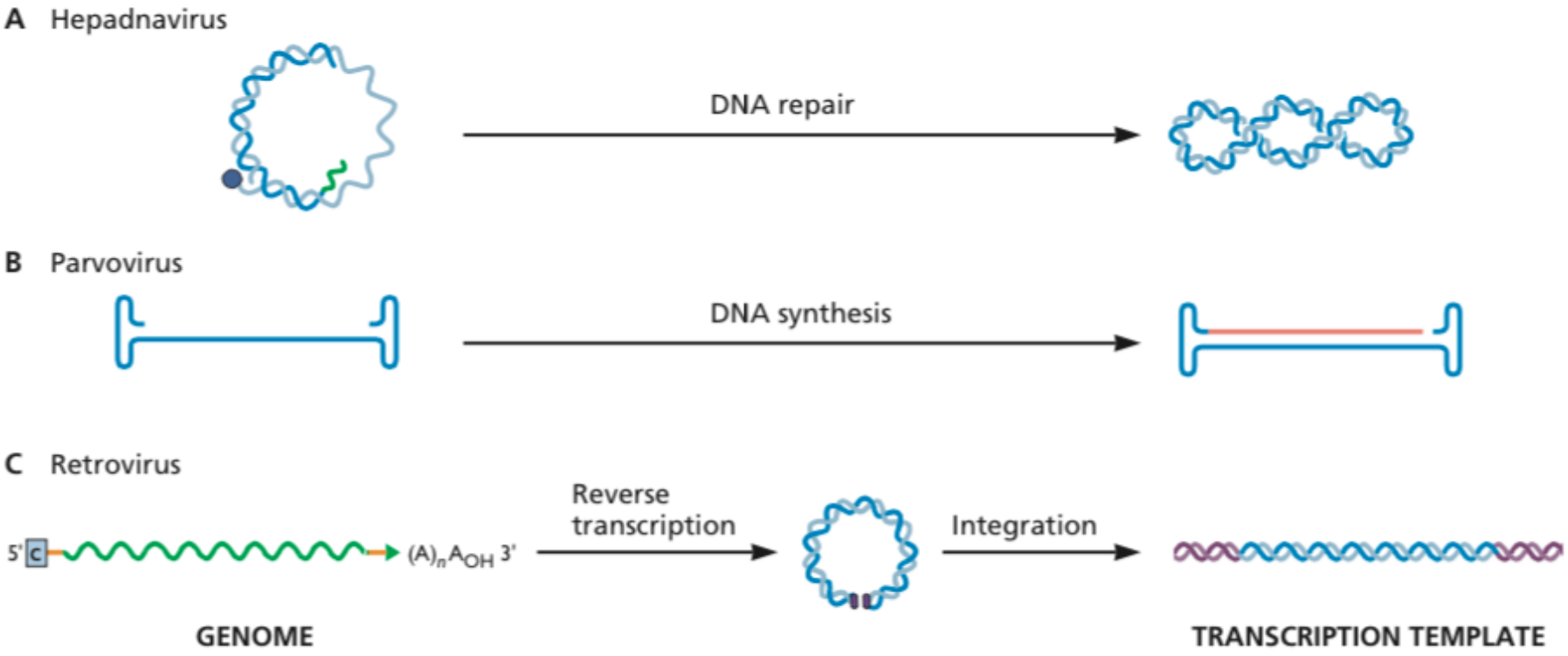
Conversion of viral genomes to templates for transcription



Which viral genomes do not need conversion?

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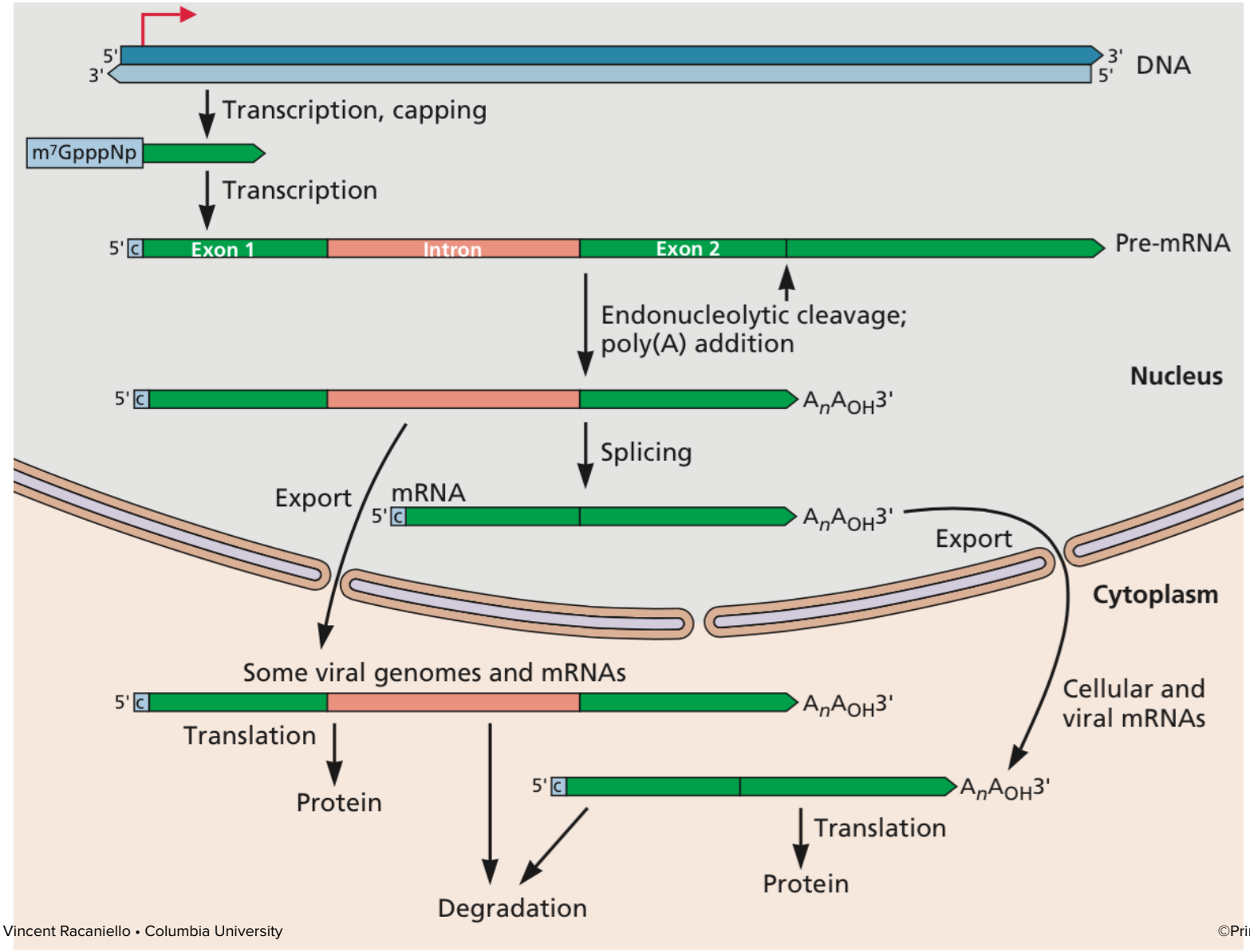

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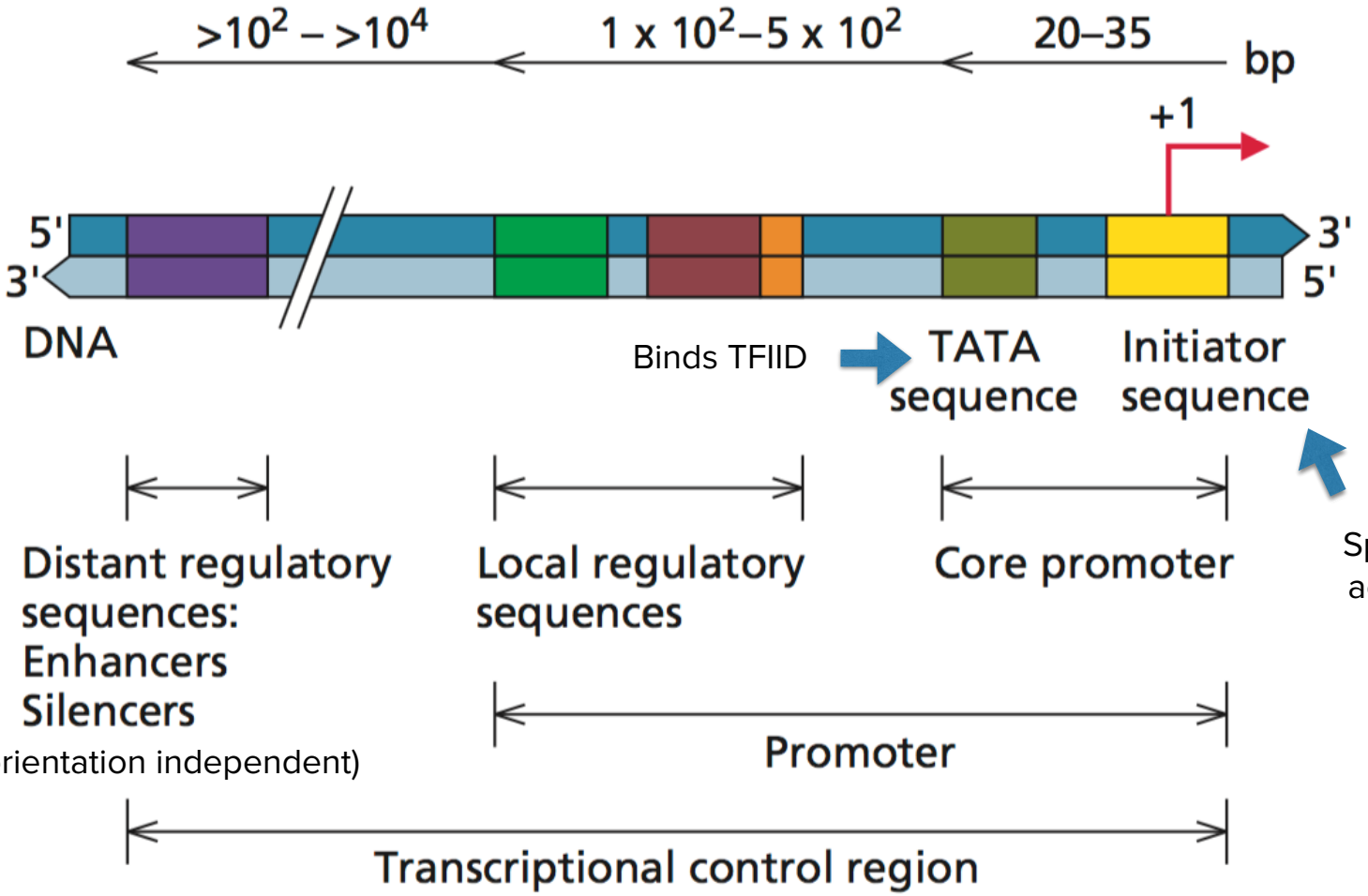
Transcription is regulated



(Specific DNA sequences that bind proteins)

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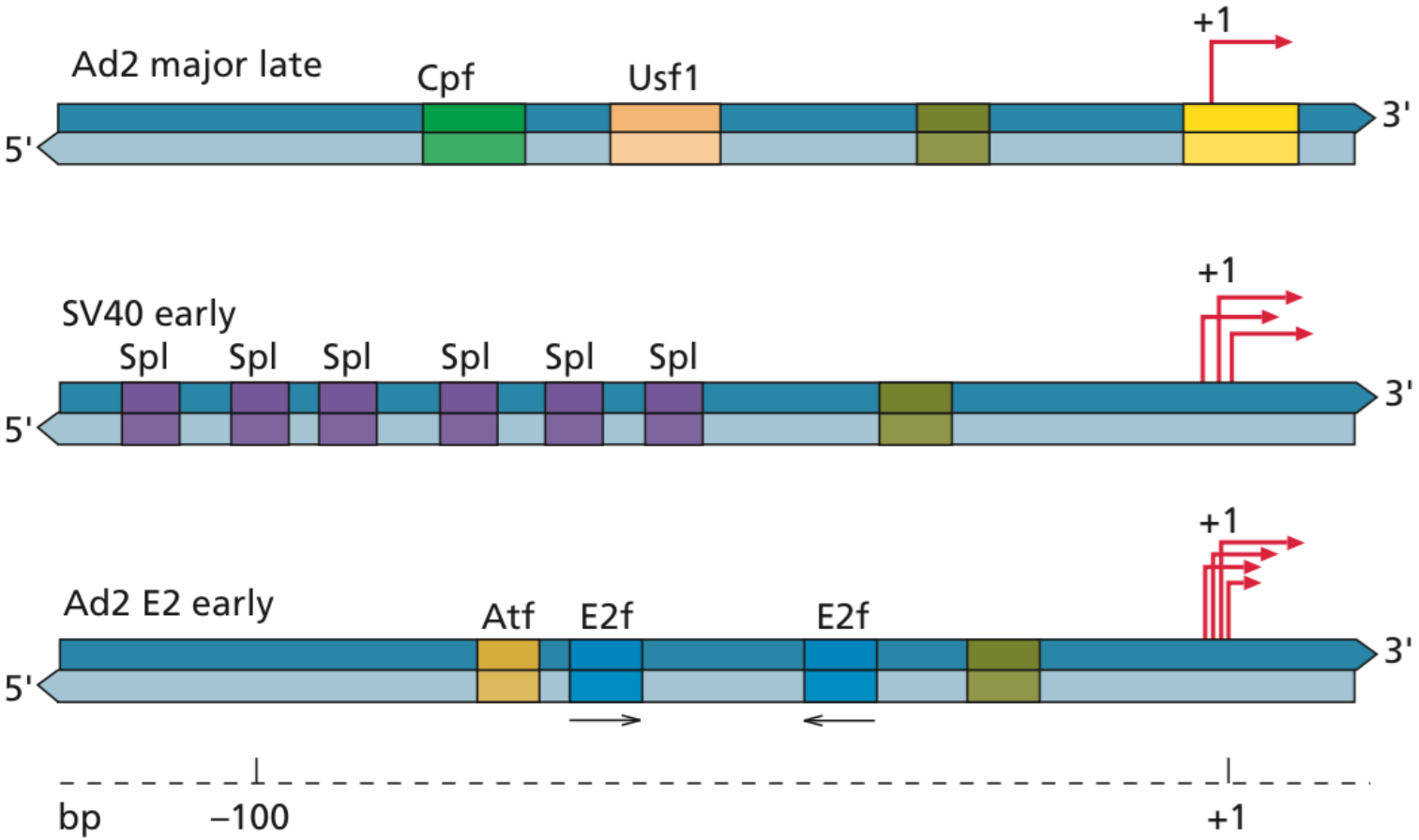
Regulatory sequences in transcriptional control regions



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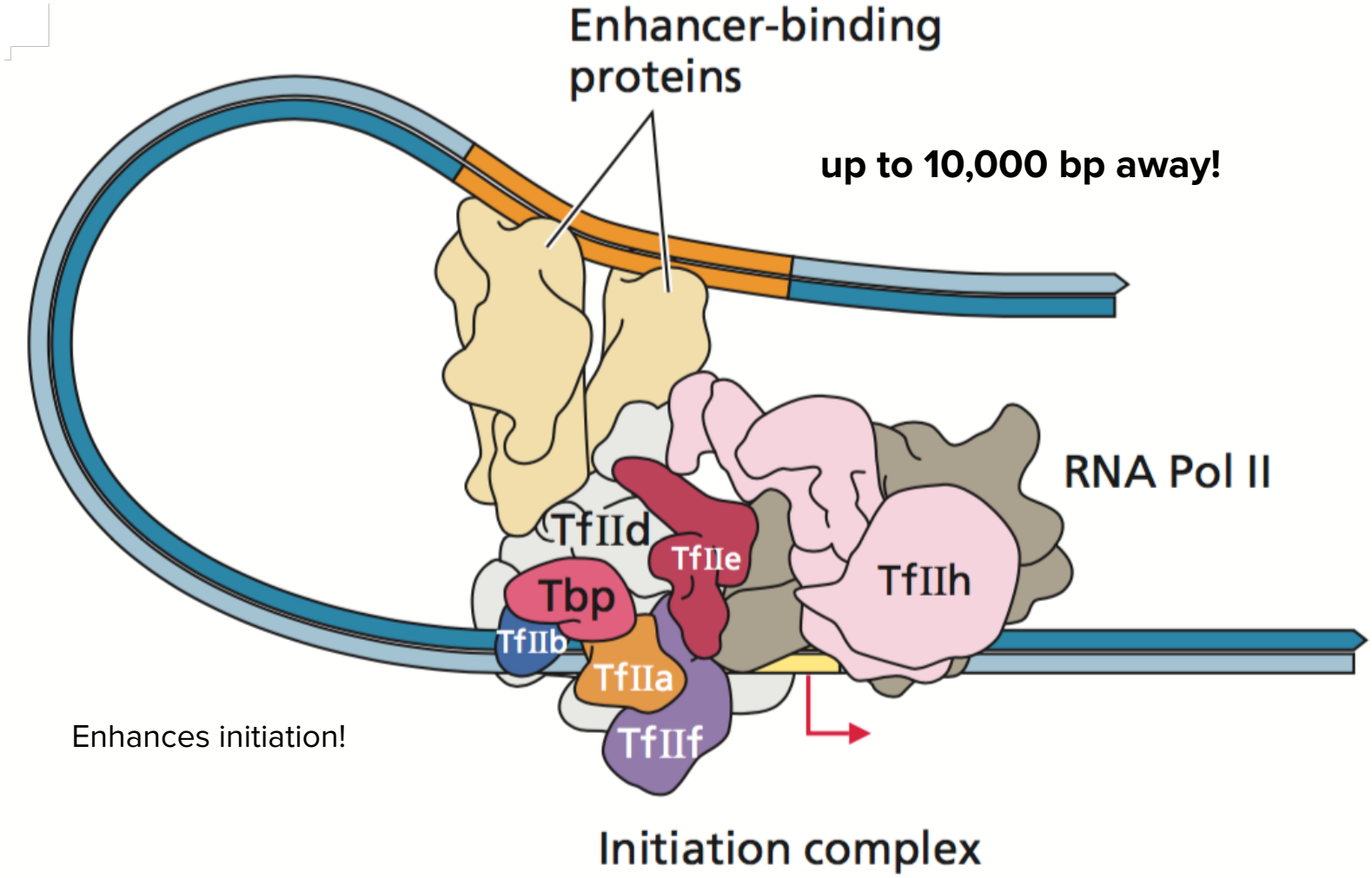

Enhances initiation!

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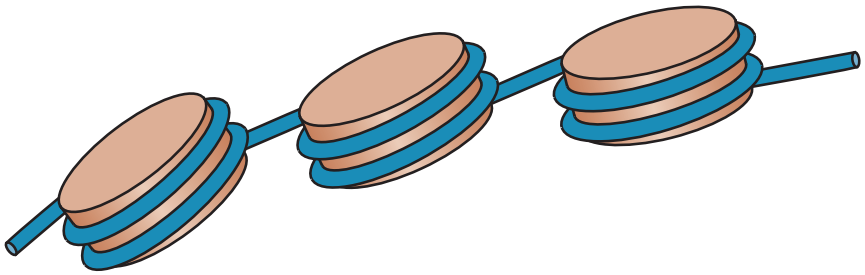
Proteins that regulate transcription

- 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 of nucleosomal templates



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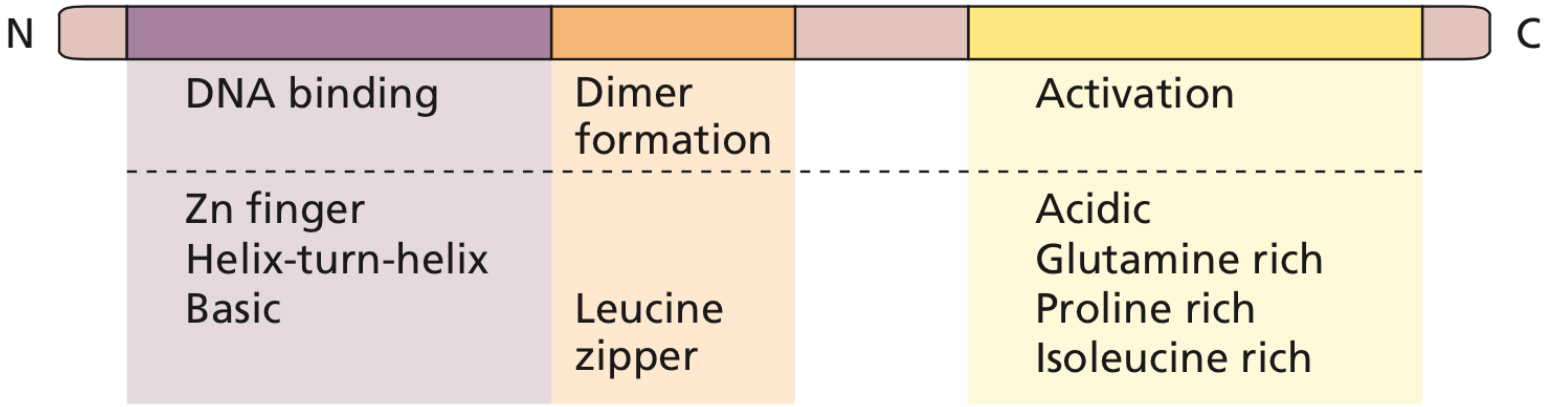
Sequence-specific transcriptional activators



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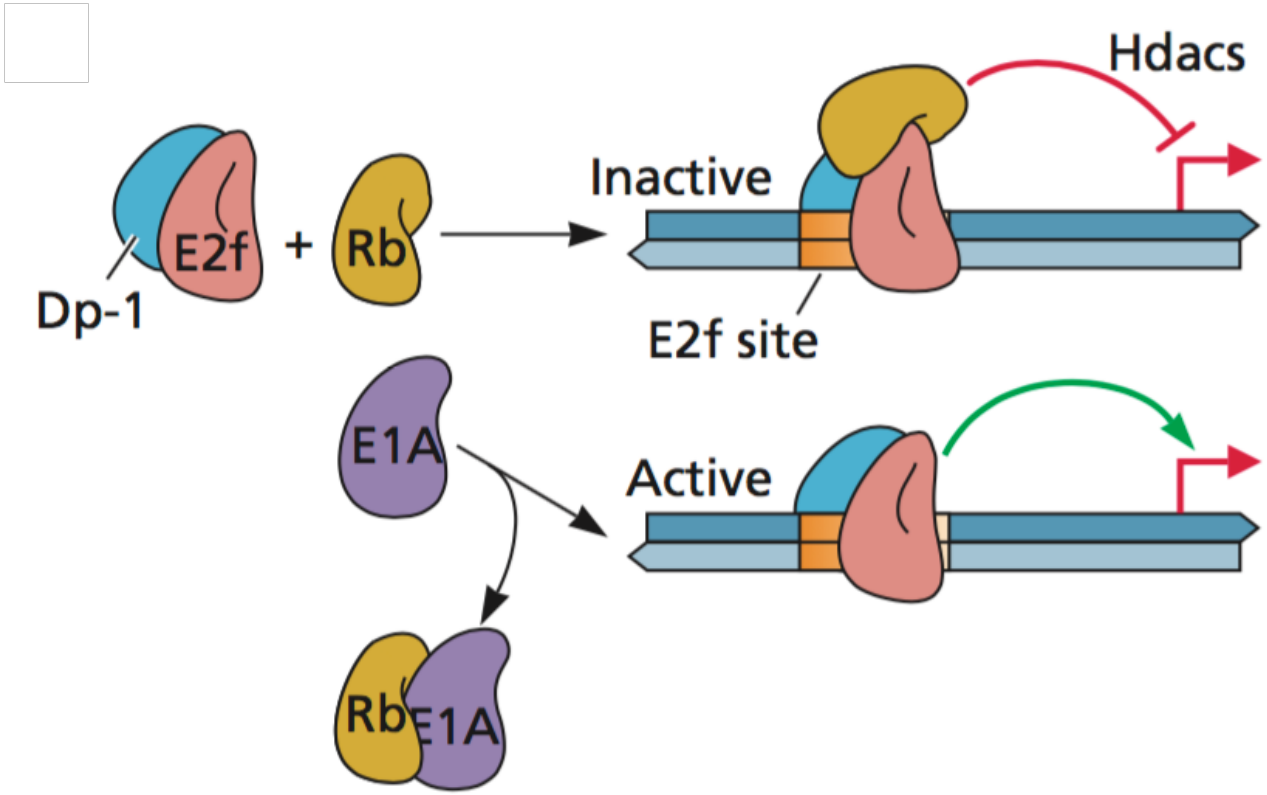
Stimulation of transcription by Ad E1A proteins



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

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

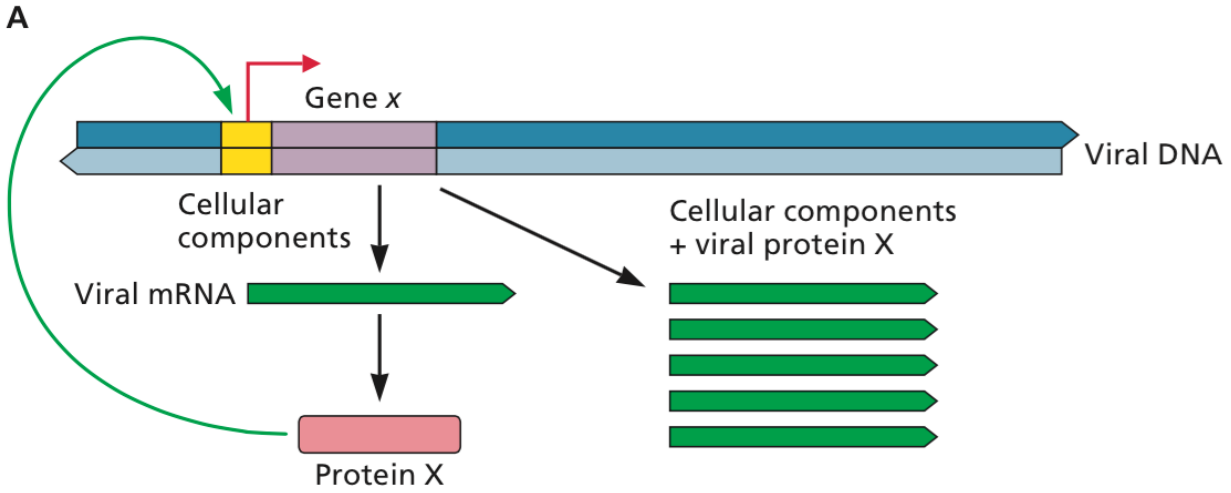
A. Membrane fusion
B. Transcription
C. DNA replication
D. Protein synthesis
E. All of the above

Strategies of transcription of viral DNA

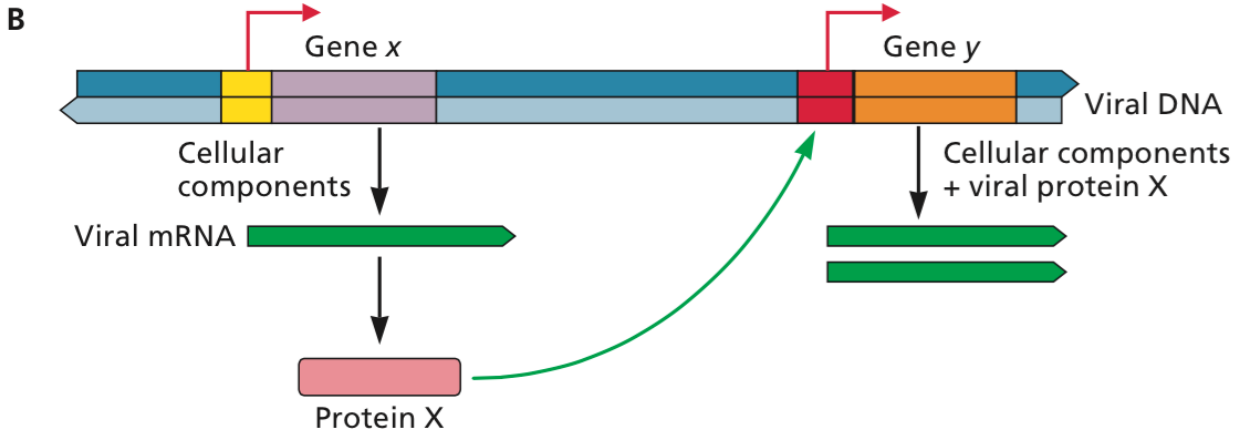
Origin of transcriptional components	Virus
Host only	Simple retroviruses
Host plus one viral protein that regulates transcription	Complex retroviruses, parvoviruses, papillomaviruses, polyomaviruses
Host plus >1 viral protein that stimulate transcription	Adenoviruses, herpesviruses
Viral	Poxviruses

Regulation of transcription by viral proteins

Positive autoregulatory loop (may also be negative)



Cascade regulation



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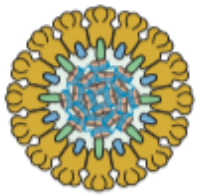
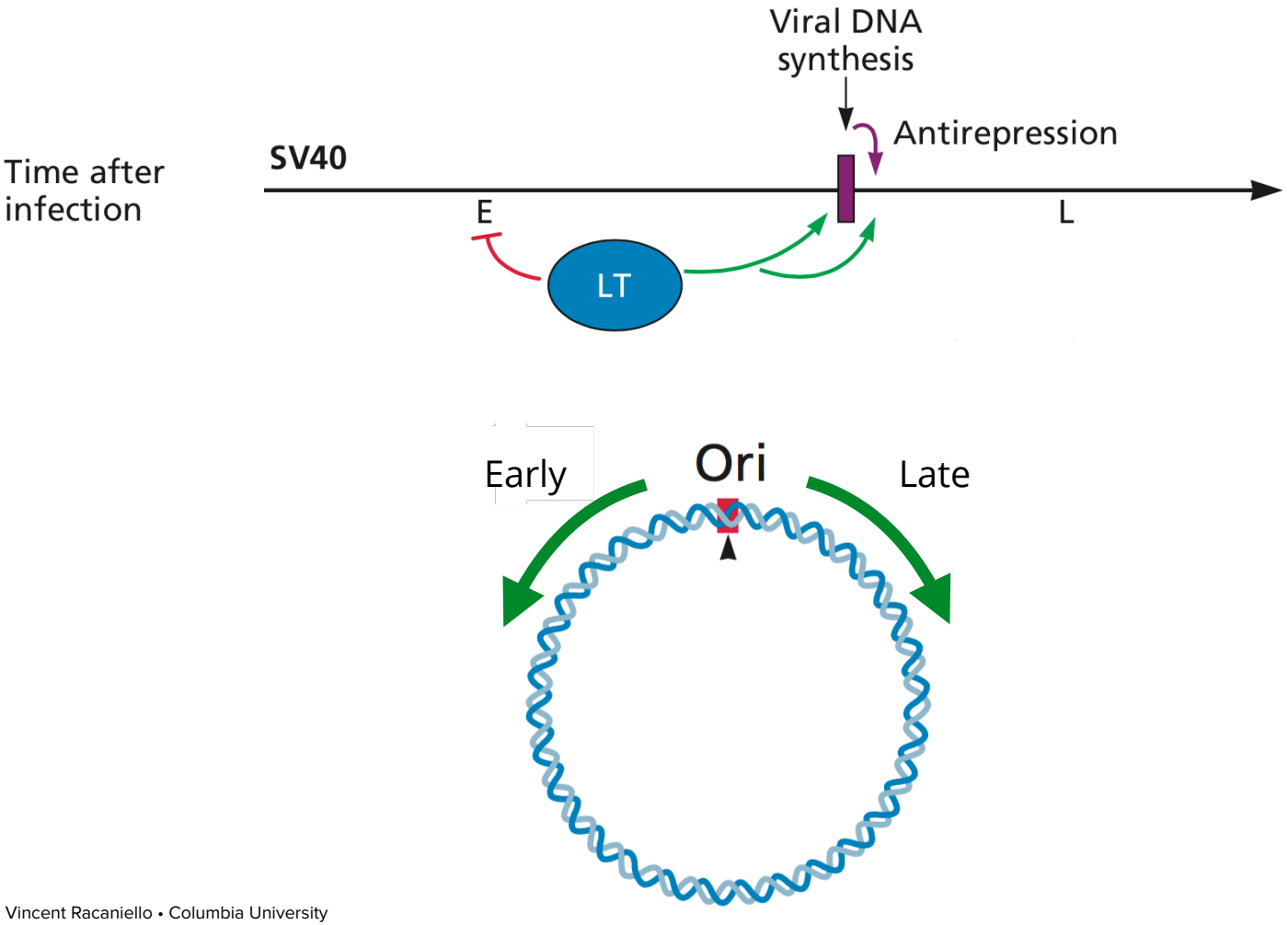
Viral transcriptional programs: SV40





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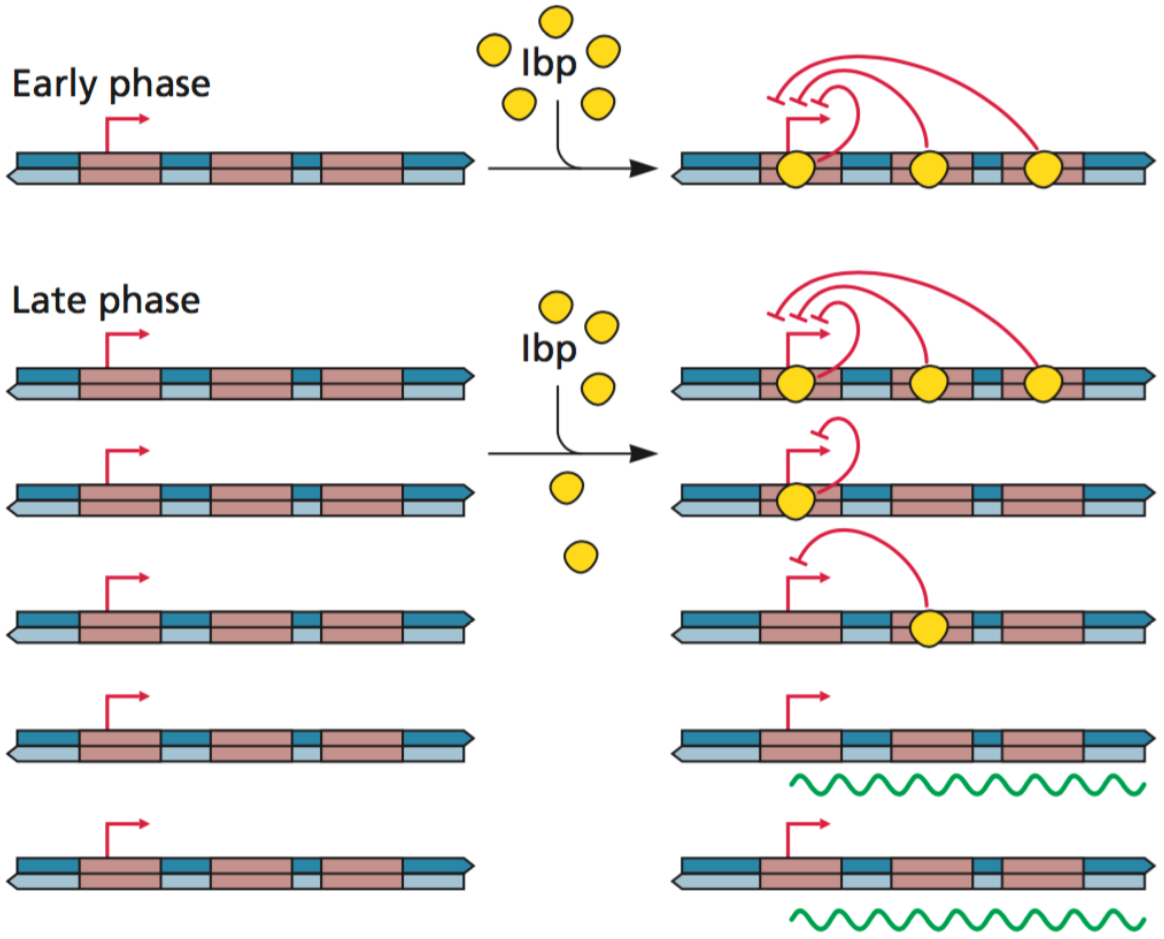
Regulation of SV40 late promoter by cellular repressors



ibp = initiator binding protein

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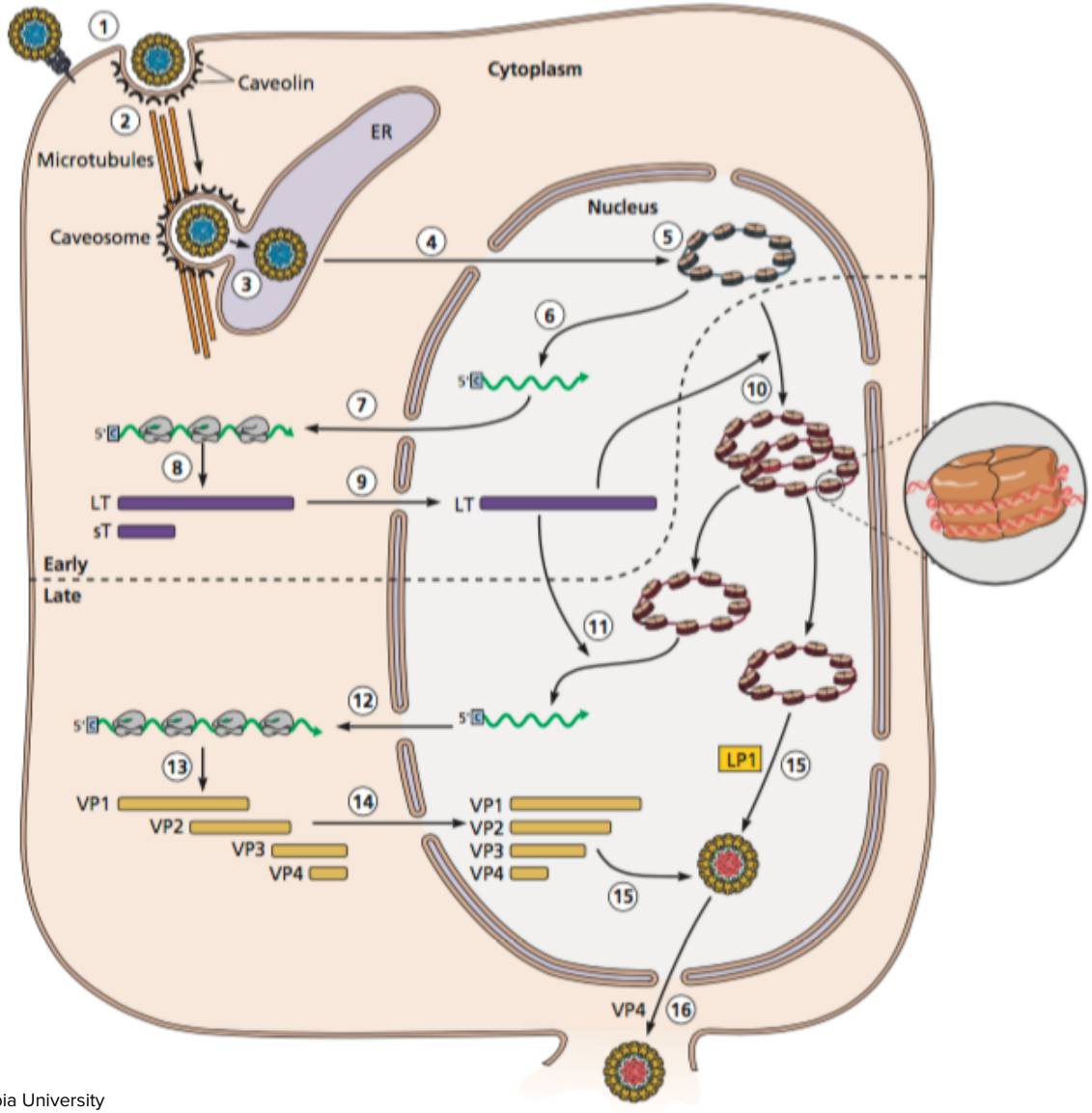

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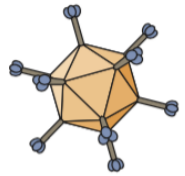
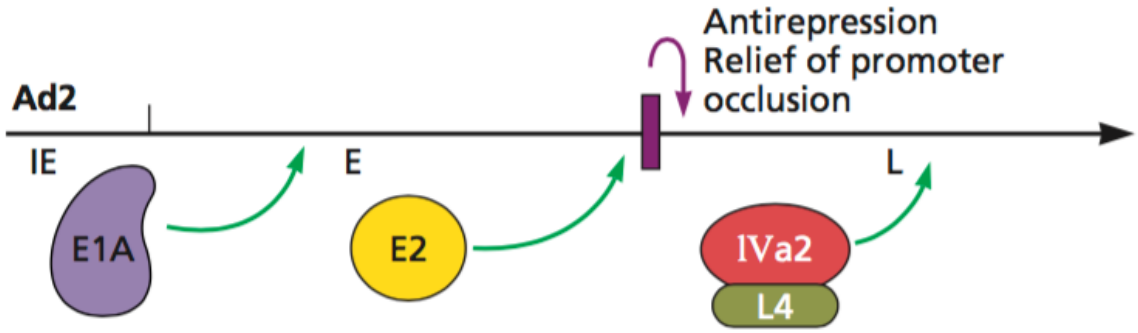
Adenovirus transcriptional regulation





- 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

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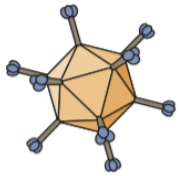
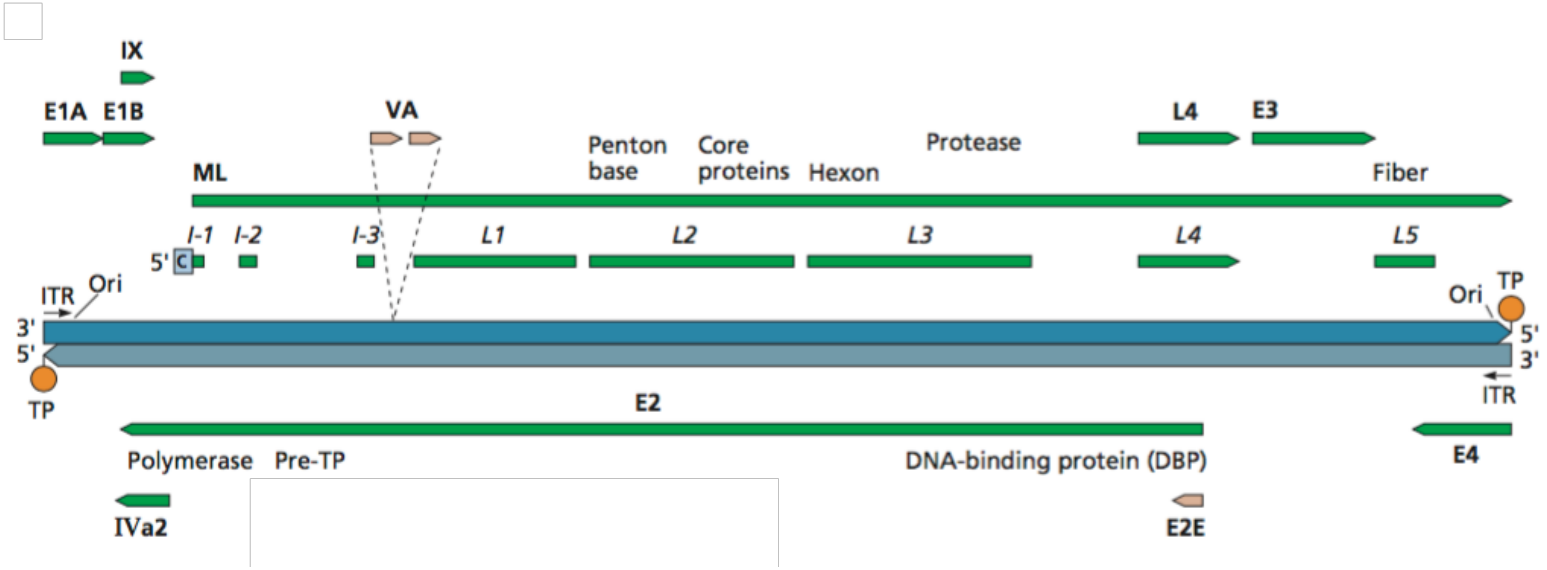
Adenovirus transcription units





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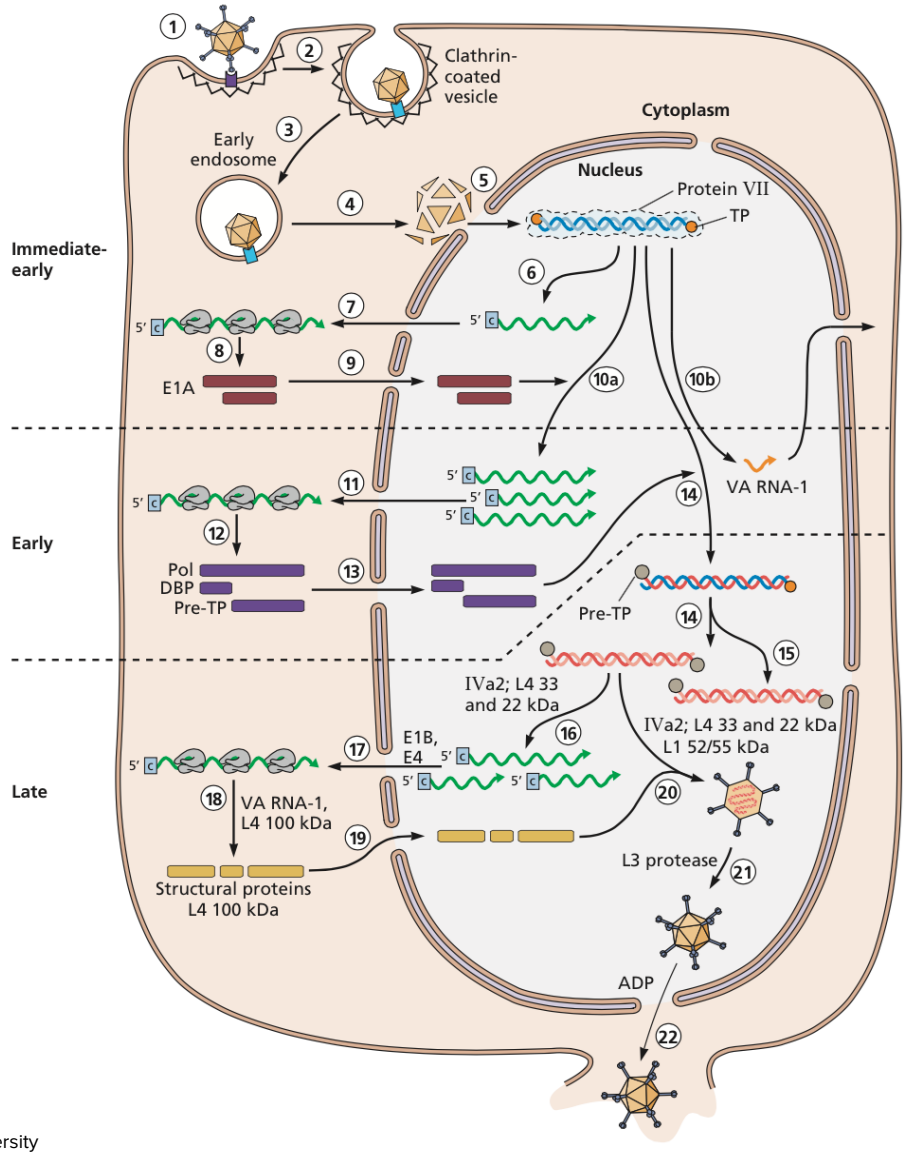

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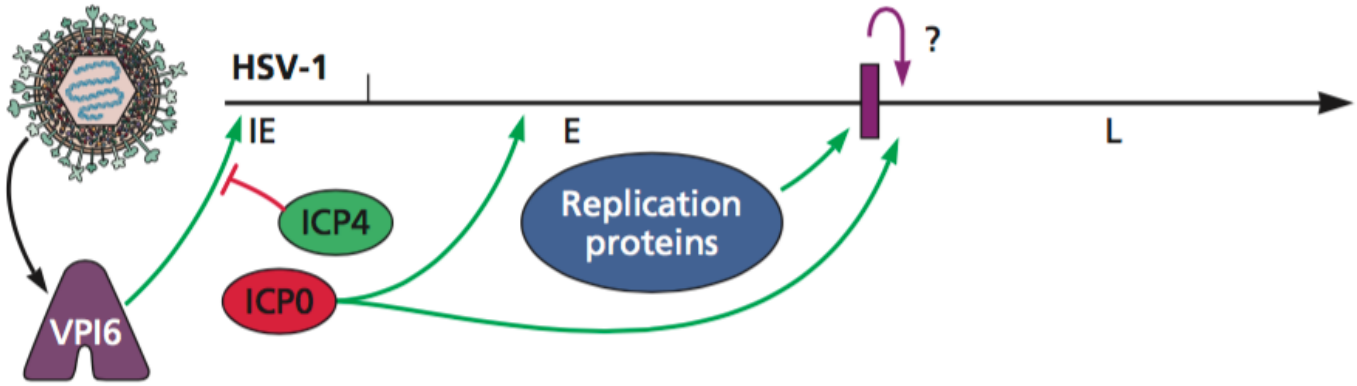
Herpesvirus transcriptional programs



- Initiated by VP16, a virion associated protein (differs from Py, Ad)
- Activates IE transcription
- 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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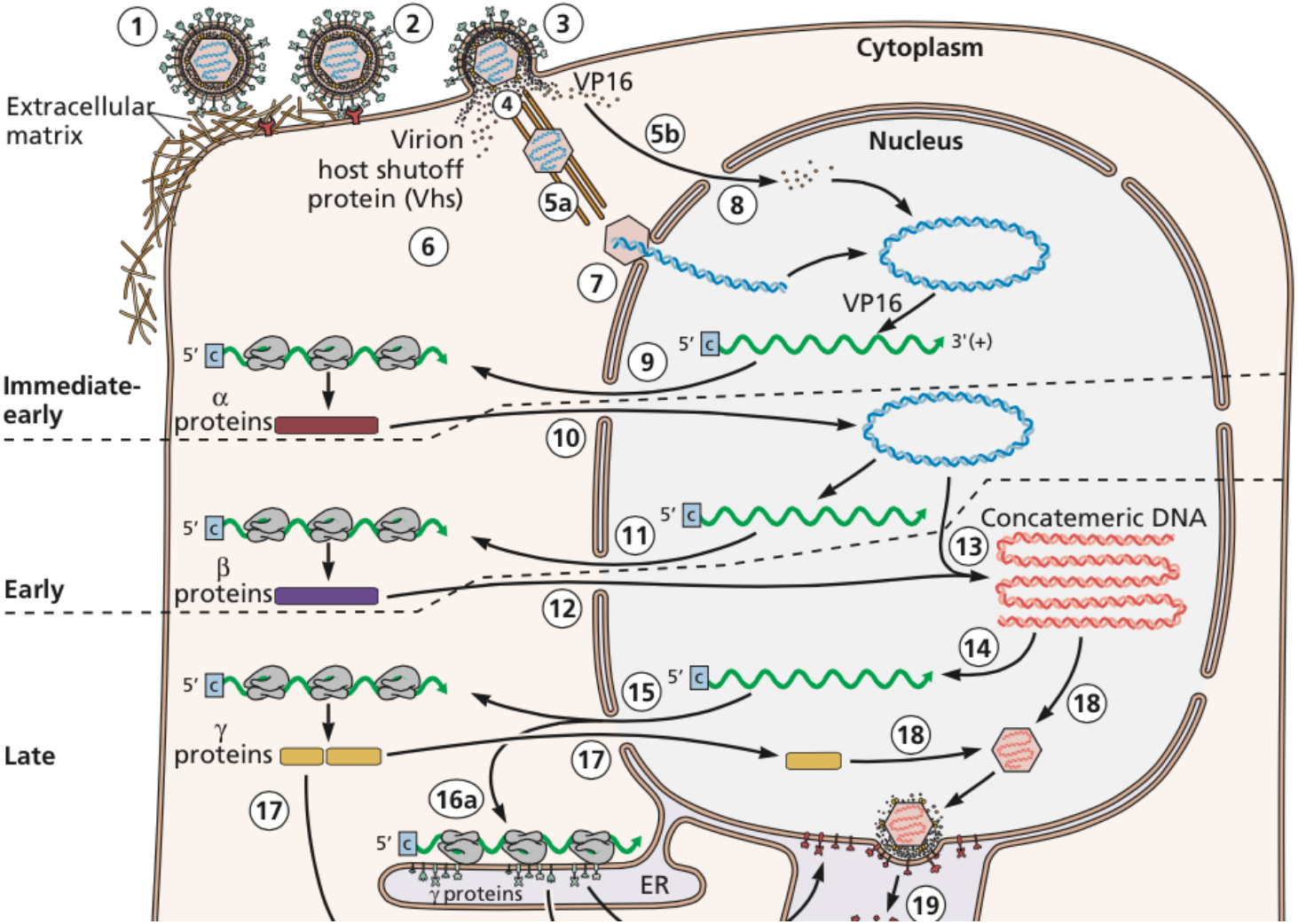

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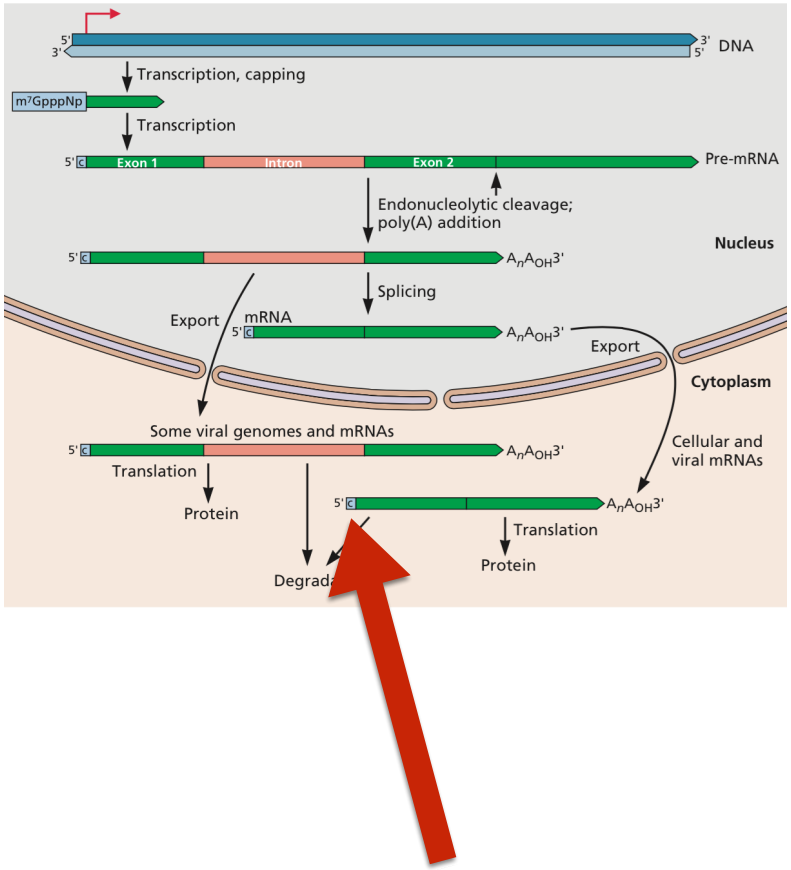
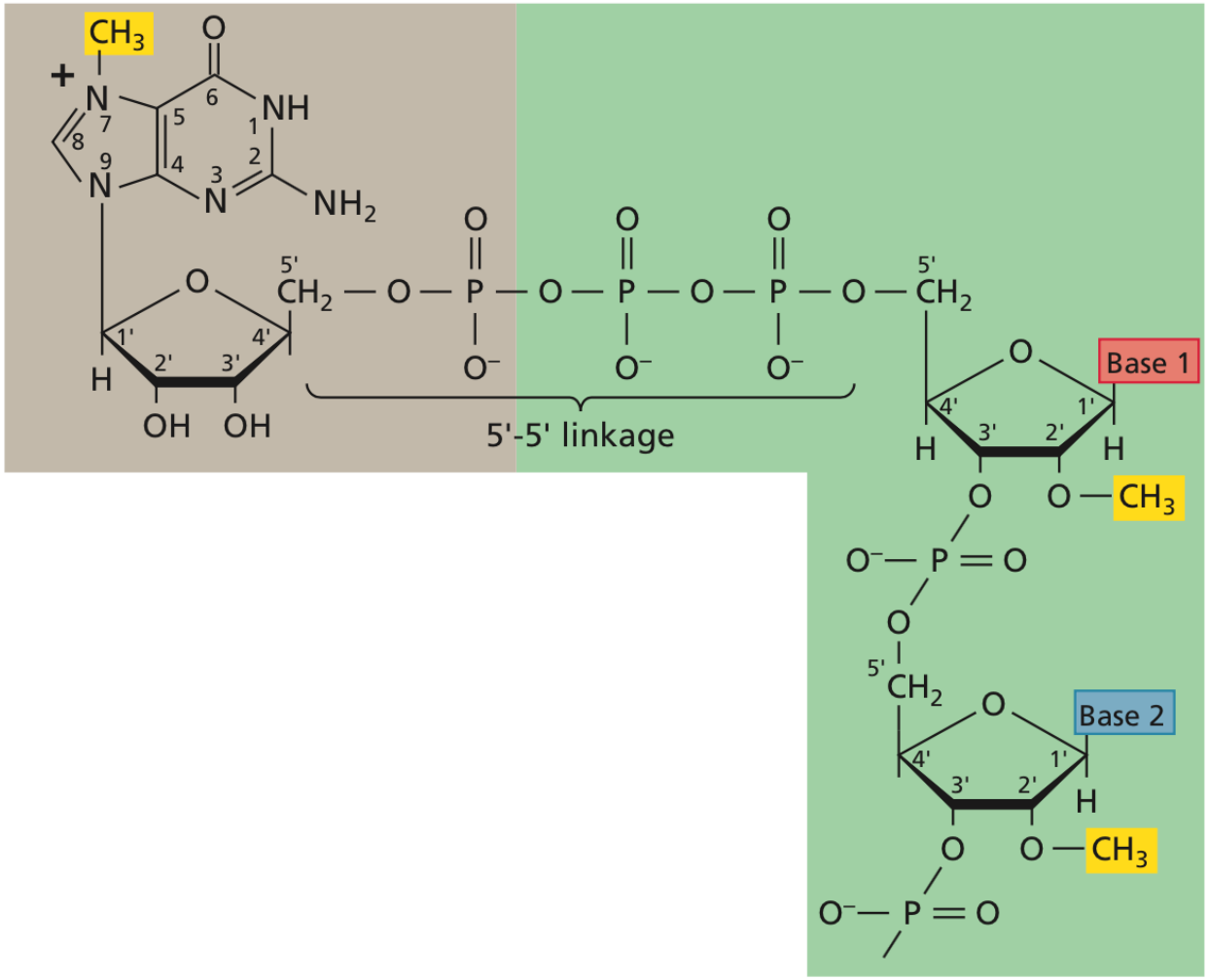
Adenovirus E1A protein stimulating the expression of adenovirus E2 protein which then stimulates the expression of adenovirus IVa2 & L4 protein is an example of:

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

Modification of mRNA: 5’-cap structure





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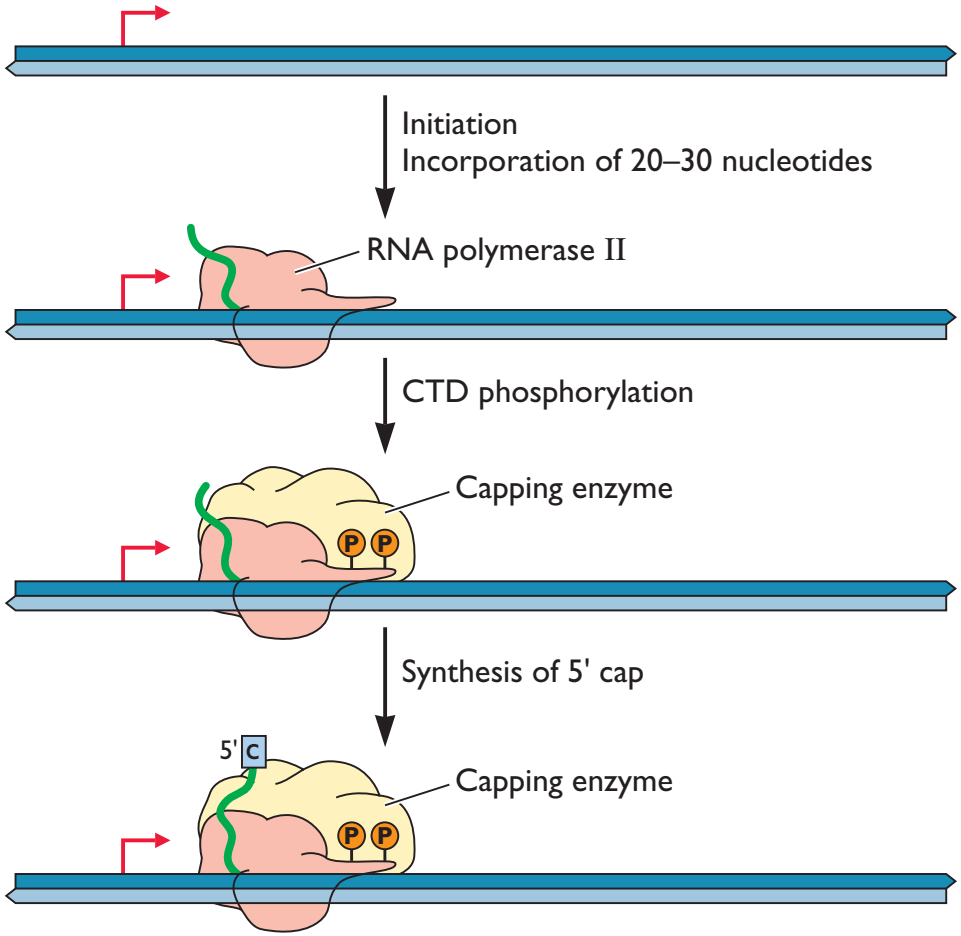
Co-transcriptional capping



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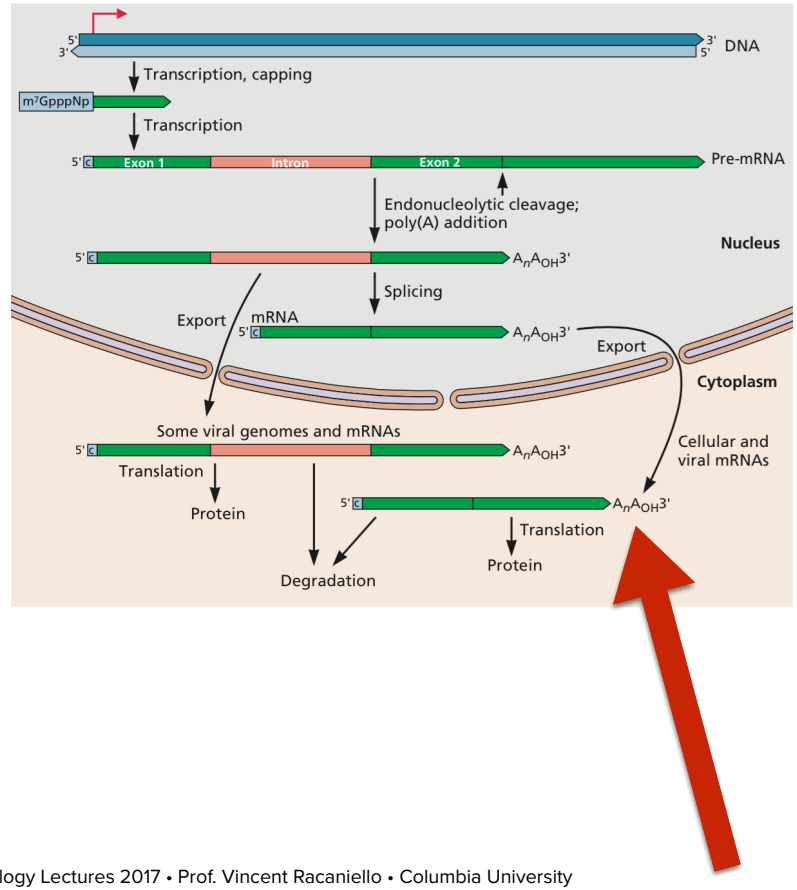
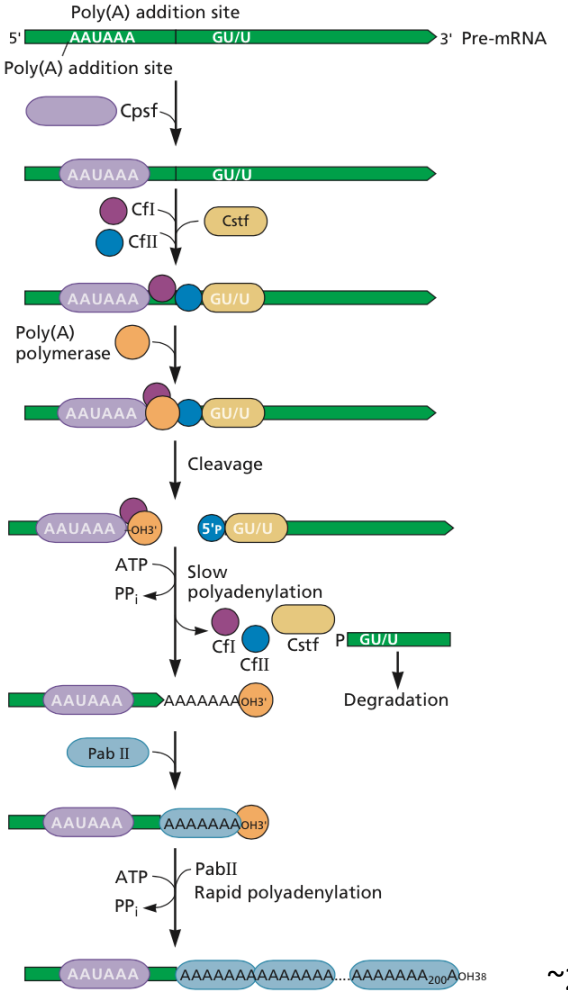
Modification of mRNA: Cleavage and polyadenylation





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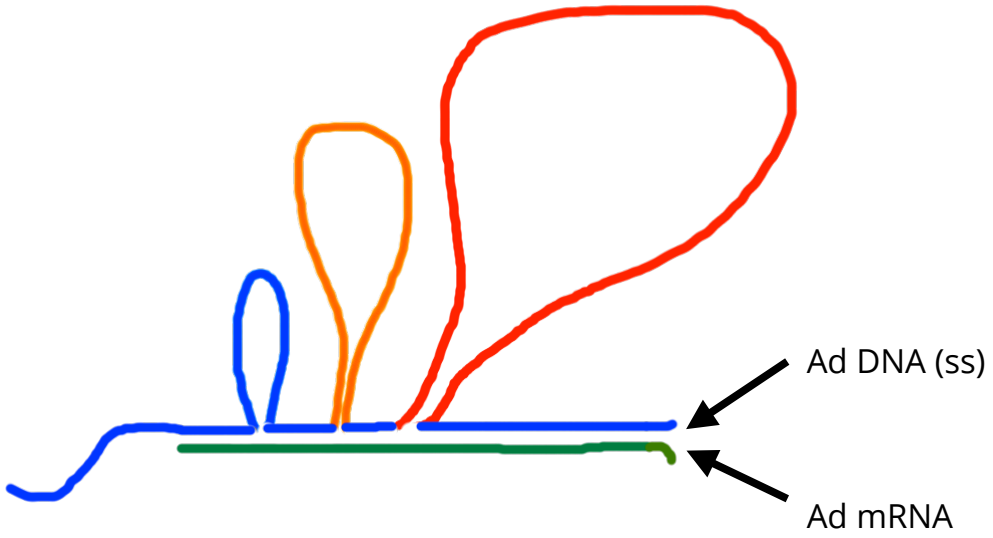
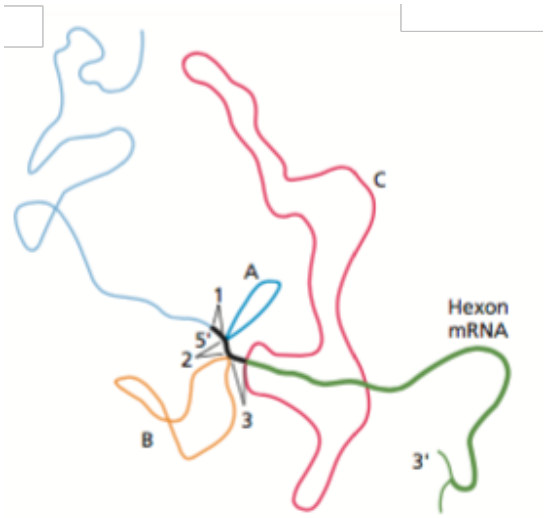
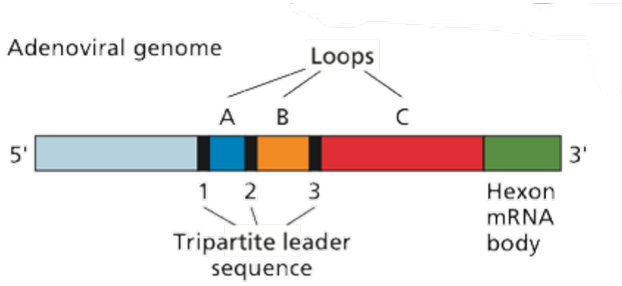
Addition of poly(A) to viral mRNAs

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







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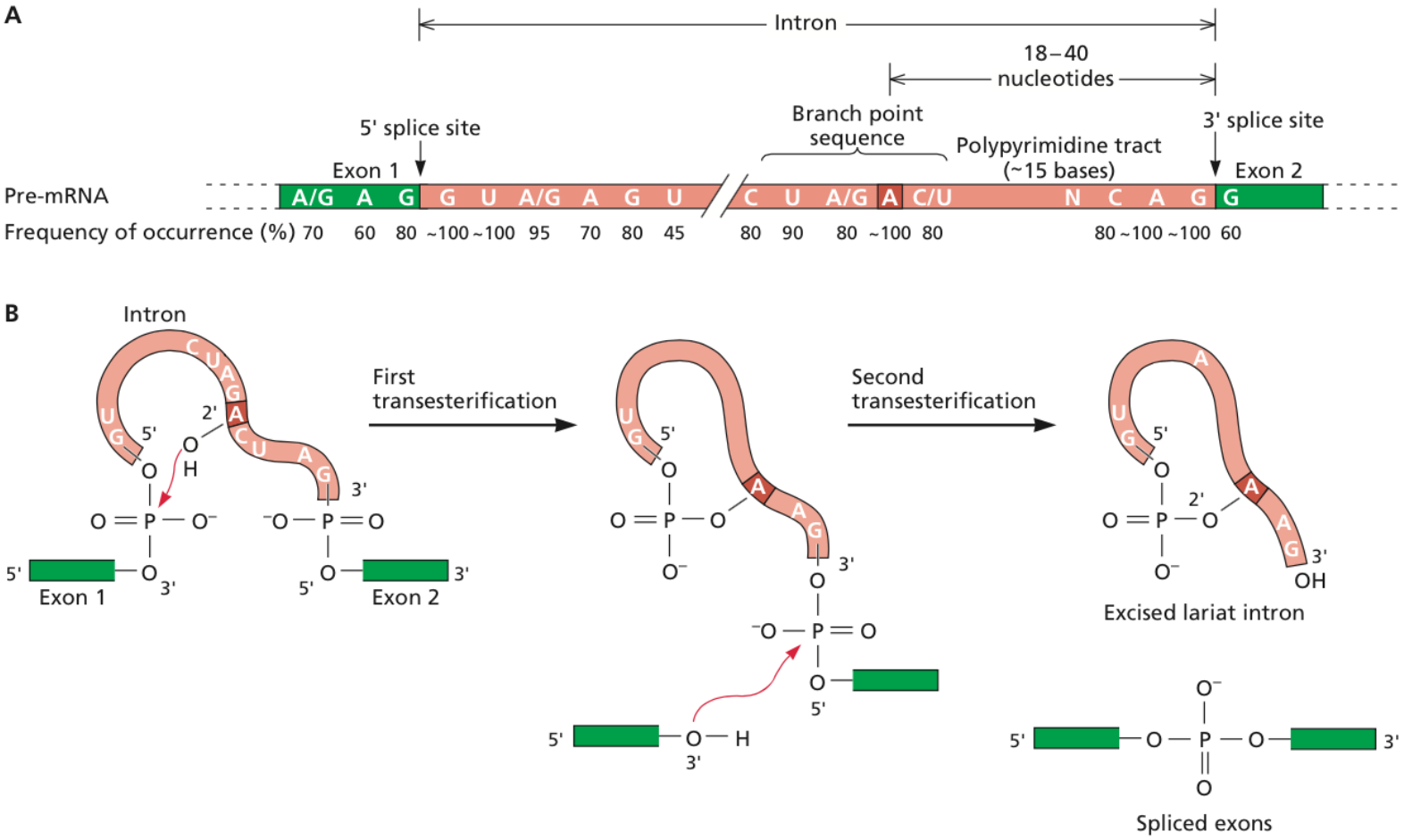
Splicing of pre-mRNA



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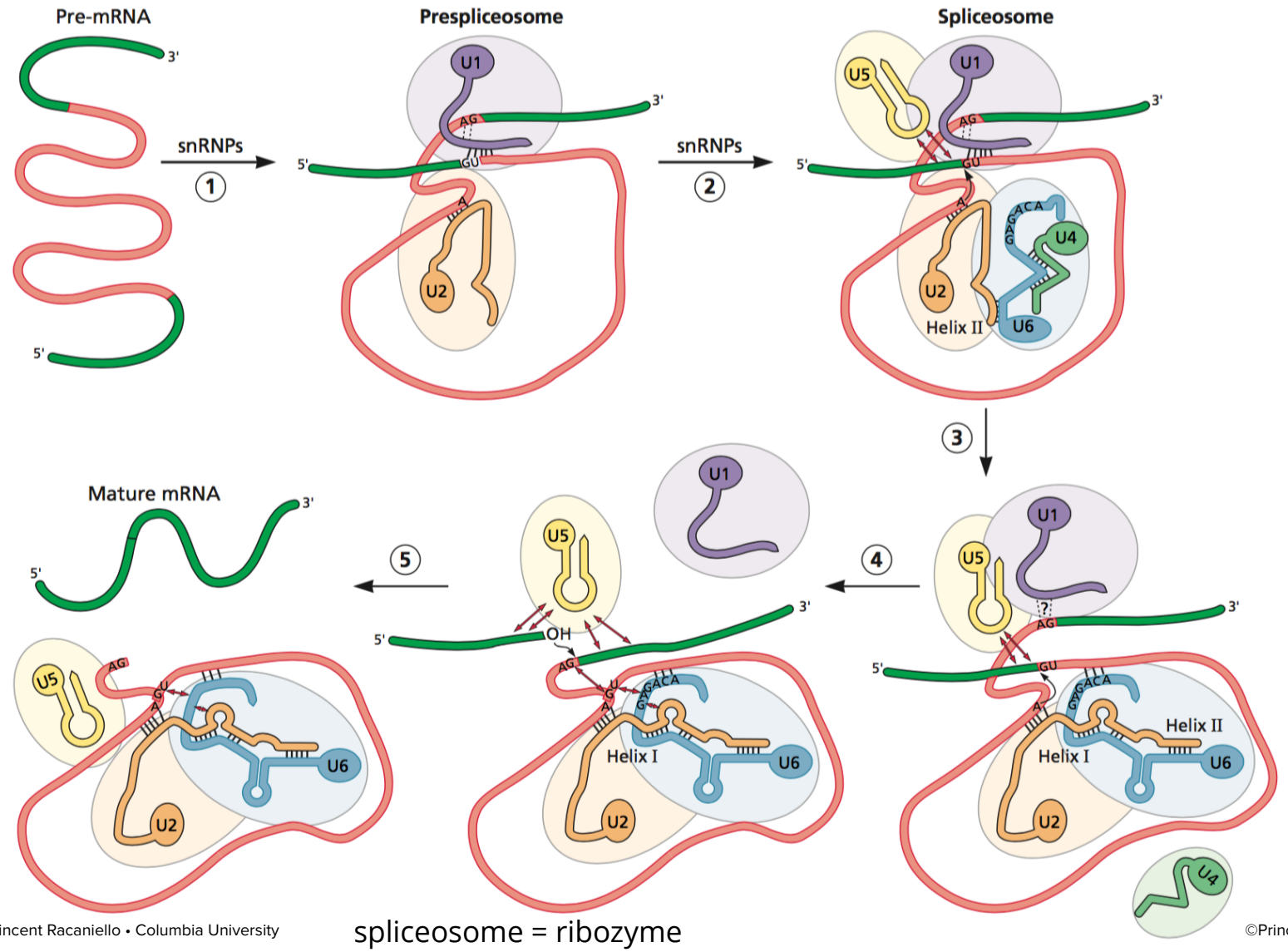

spliceosome = ribozyme

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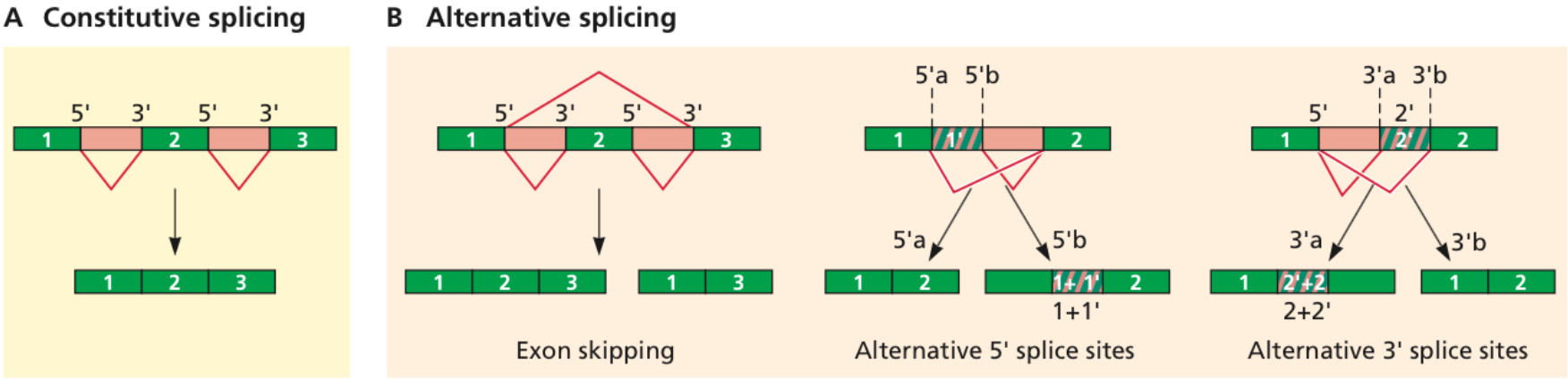
Constitutive and alternative splicing



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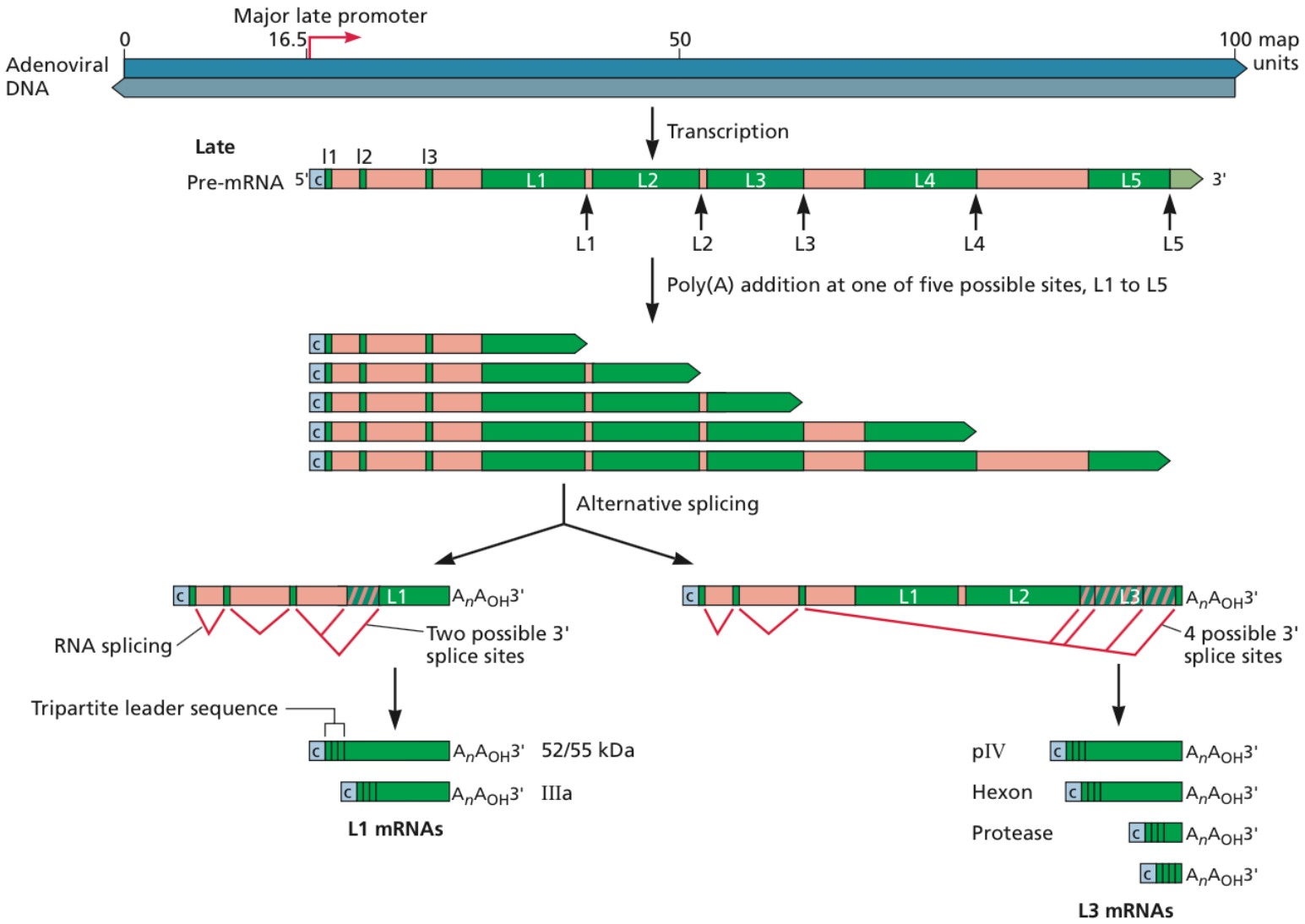

Viral proteins can regulate alternative splicing

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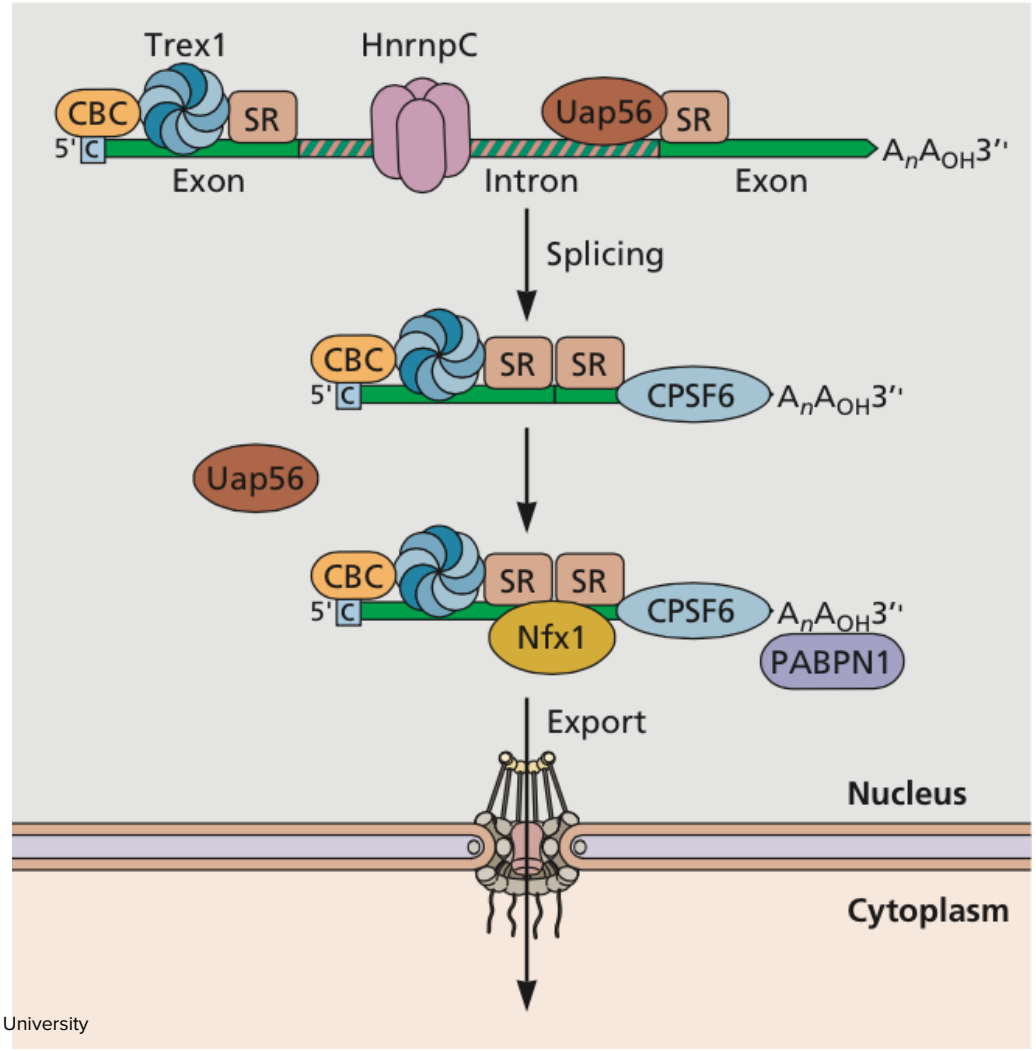
Which statement about polyadenylation of DNA virus mRNAs is correct?

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

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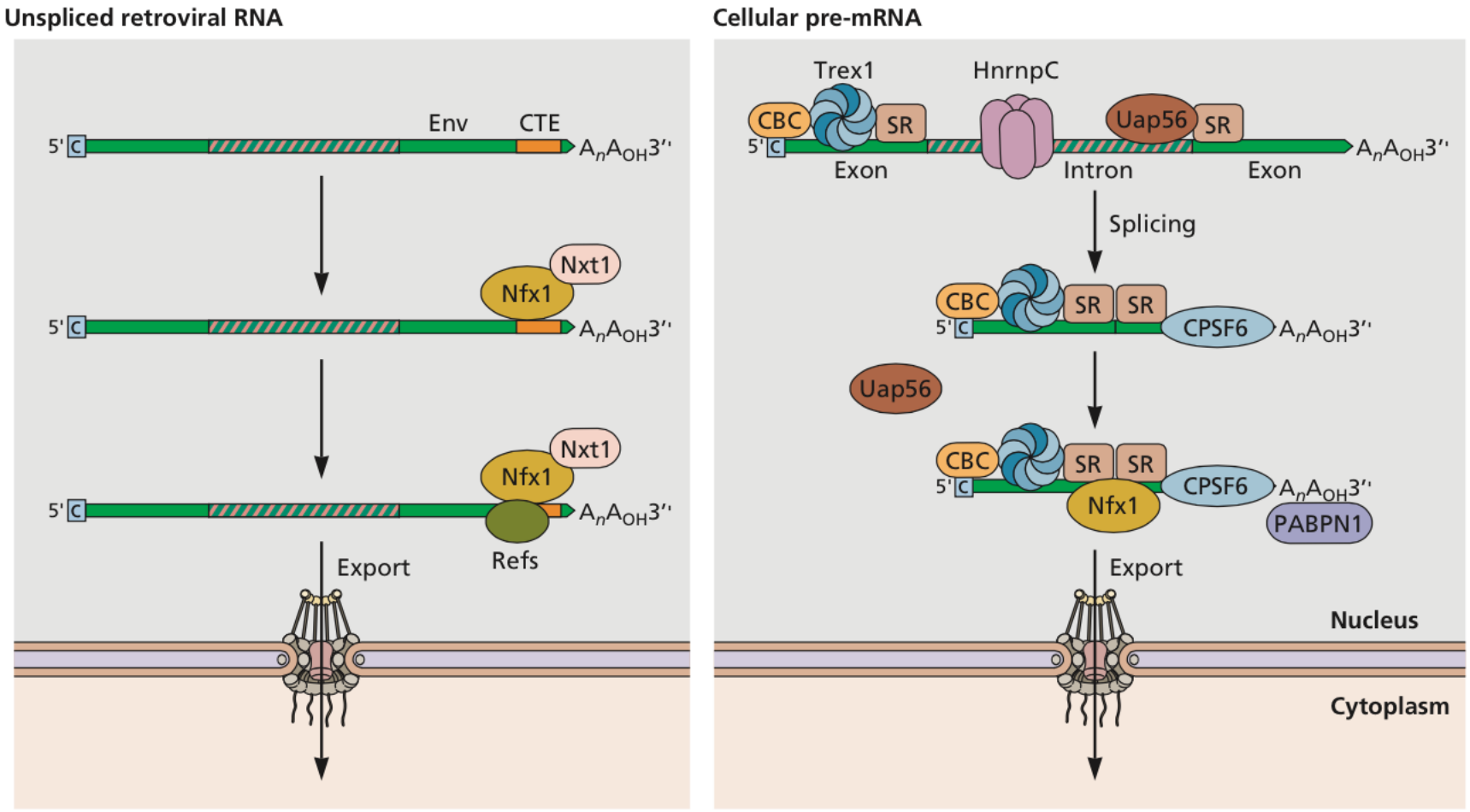
Export of unspoiled retroviral mRNA



CTE = Constitutive transport element

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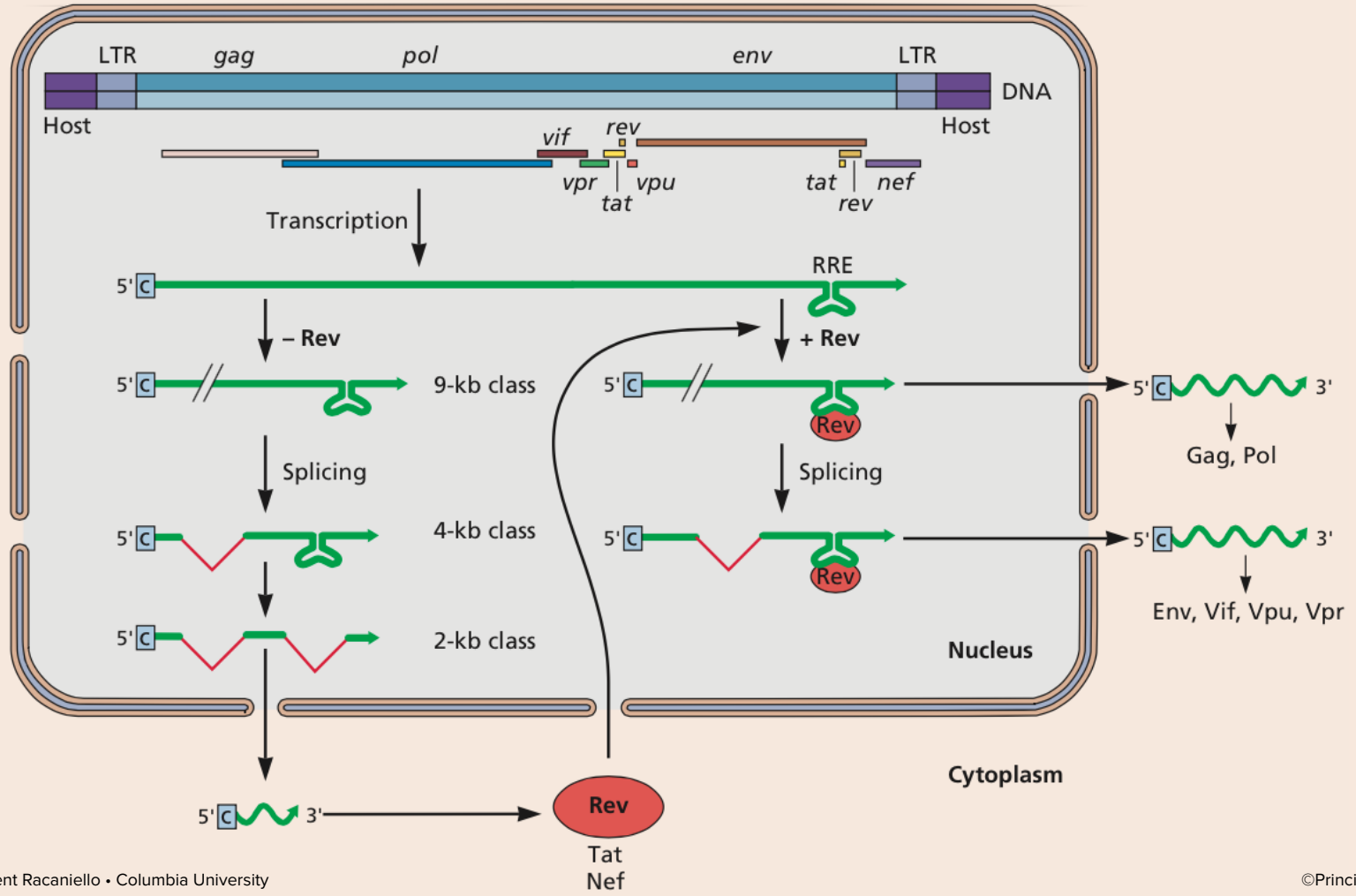
Rev protein regulates export of HIV mRNA



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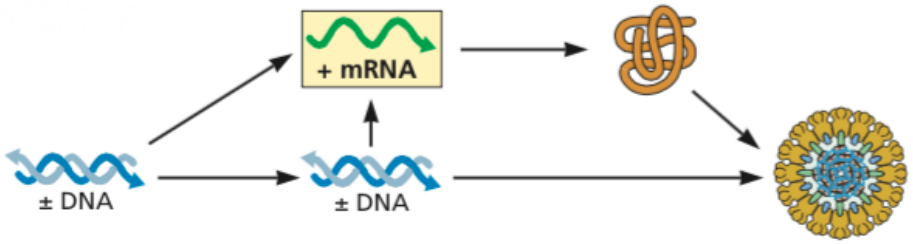
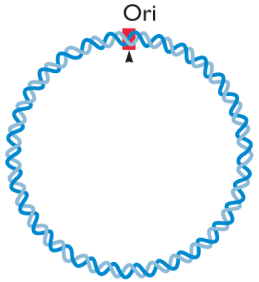
Splicing = Value added

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

DNA genomes

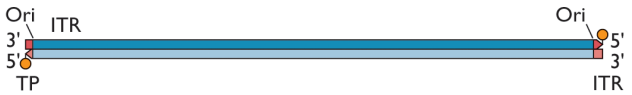


***Polyomaviridae* (5 kbp)**

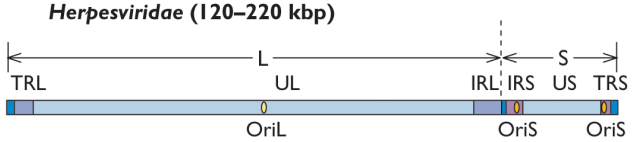


***Circoviridae* (1.7–2.2 kb)**

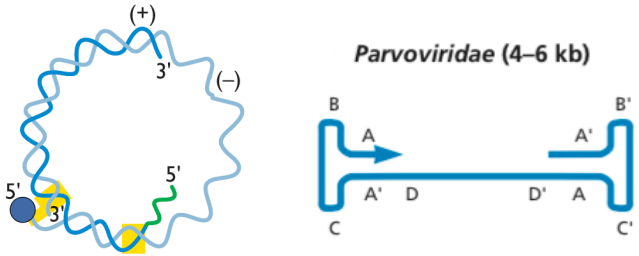
***Adenoviridae* (36–48 kbp)**



***Herpesviridae* (120–220 kbp)**



***Parvoviridae* (4–6 kb)**



***Poxviridae* (130–375 kbp)**

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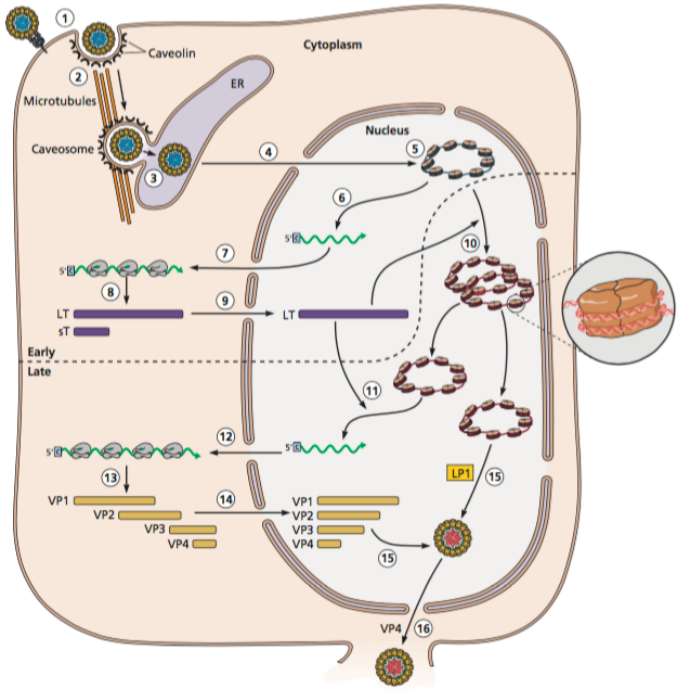
Why does transcription occur first for DNA viruses?

Viral DNA replication always requires synthesis of at least one viral protein, sometimes many - hence it is always delayed after infection



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