Transcription and RNA Processing

Lecture 8
Virology W3310/4310
Spring 2013

Viruses are Informative

- Control signals
- Nature of a promoter
- What an enhancer is
- What introns and exons are
- How RNA synthesis is initiated and regulated

Paradigms for Transcription

- One of the first events following infection
- Variety of "chromosome - like" templates
  -Polyomaviridae - a regular array of nucleosomes
  -Adenovirus and Herpesvirus - chromatin-like DNA structures
  -HIV - transcribed from integrated DNA
- Regulation, expression is strictly defined

Transcription

- Regulation is primarily controlled by initiation
  - instances where elongation is a rate controlling step
- A multi-step process, other opportunities for control
- Specificity of initiation
- Termination - both polymerase and RNA are released from template

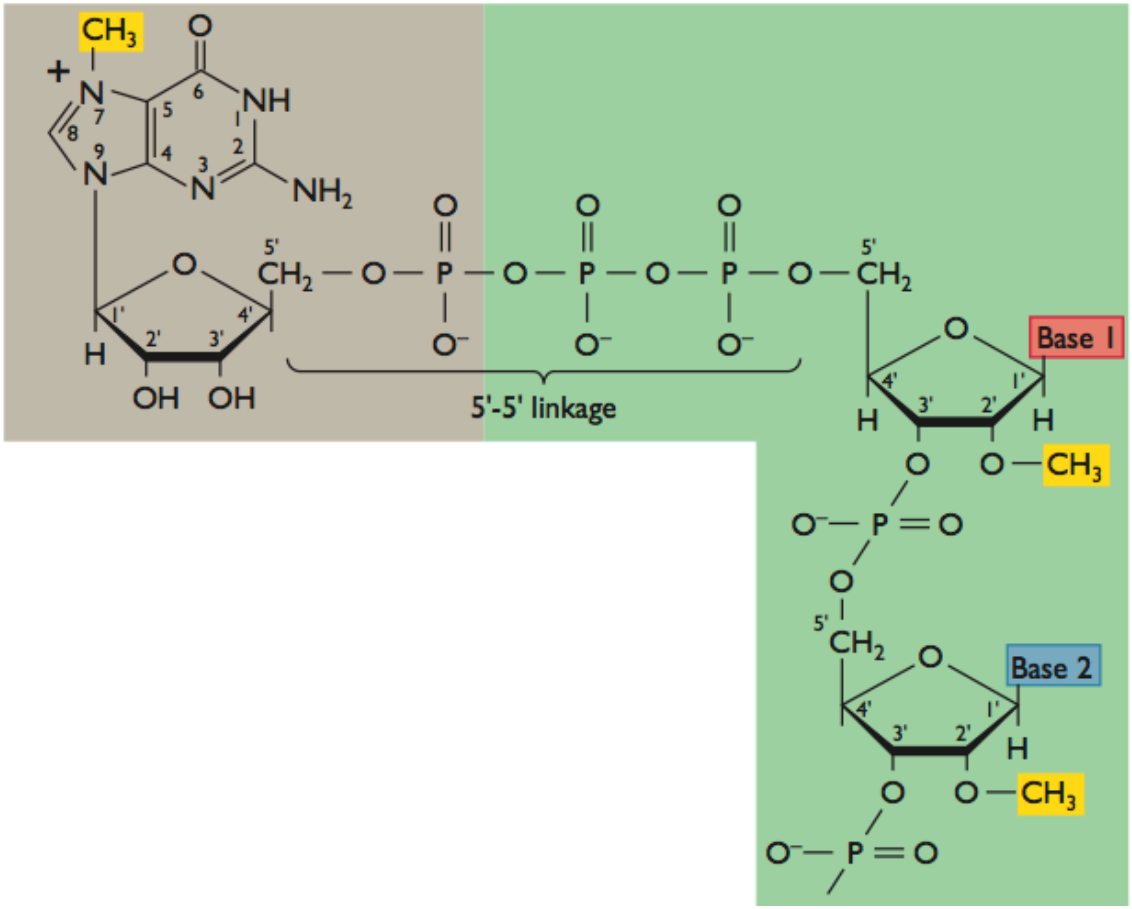
Generic Steps in Transcription

- Steps are just like DNA replication
- Promoter recognition
- Preinitiation complex formation
- Initiation
  - -site specificity
- Elongation
- Termination

What Happens to RNA Transcripts?

- Capping
- Polyadenylation
- Splicing
- Editing
- Transport
  - -becomes mRNA, gets translated
- Decay, $t_{1/2}$ is critical
- Silencing
  - -degradation
  - -inhibition of translation

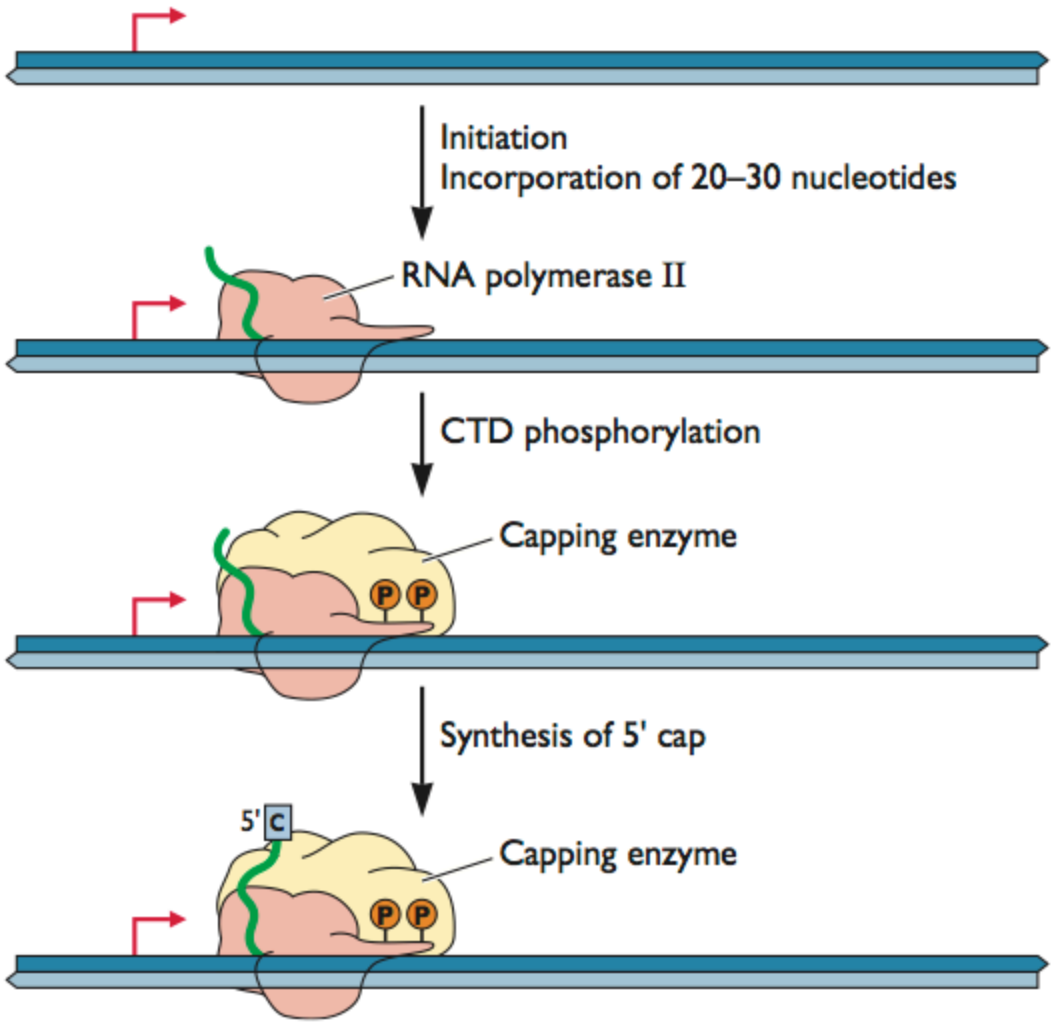
Capping



Terminal Cap Structure

- 5'-5' triphosphate linkage, capping enzyme
- 2'-O-methylation, guanine methyl transferase
- Occurs cotranscriptionally post modification of PolII

Cotranscriptional Capping



Host Polymerases

- Pol I - pre rRNA not known to be used by viruses
- Pol II - makes mRNAs and some micro RNAs
- Pol III - Adenovirus VA RNAs, EBV EBERs and some micro RNAs
- How does the virus subjugate the host?

Prerequisites for Transcription

- Adenovirus, Polyomaviruses - enter cell nucleus
- Herpesviruses -introduce virion-associated proteins
- Retroviruses - +RNA - dsDNA - Integrate

Transcriptional Programming

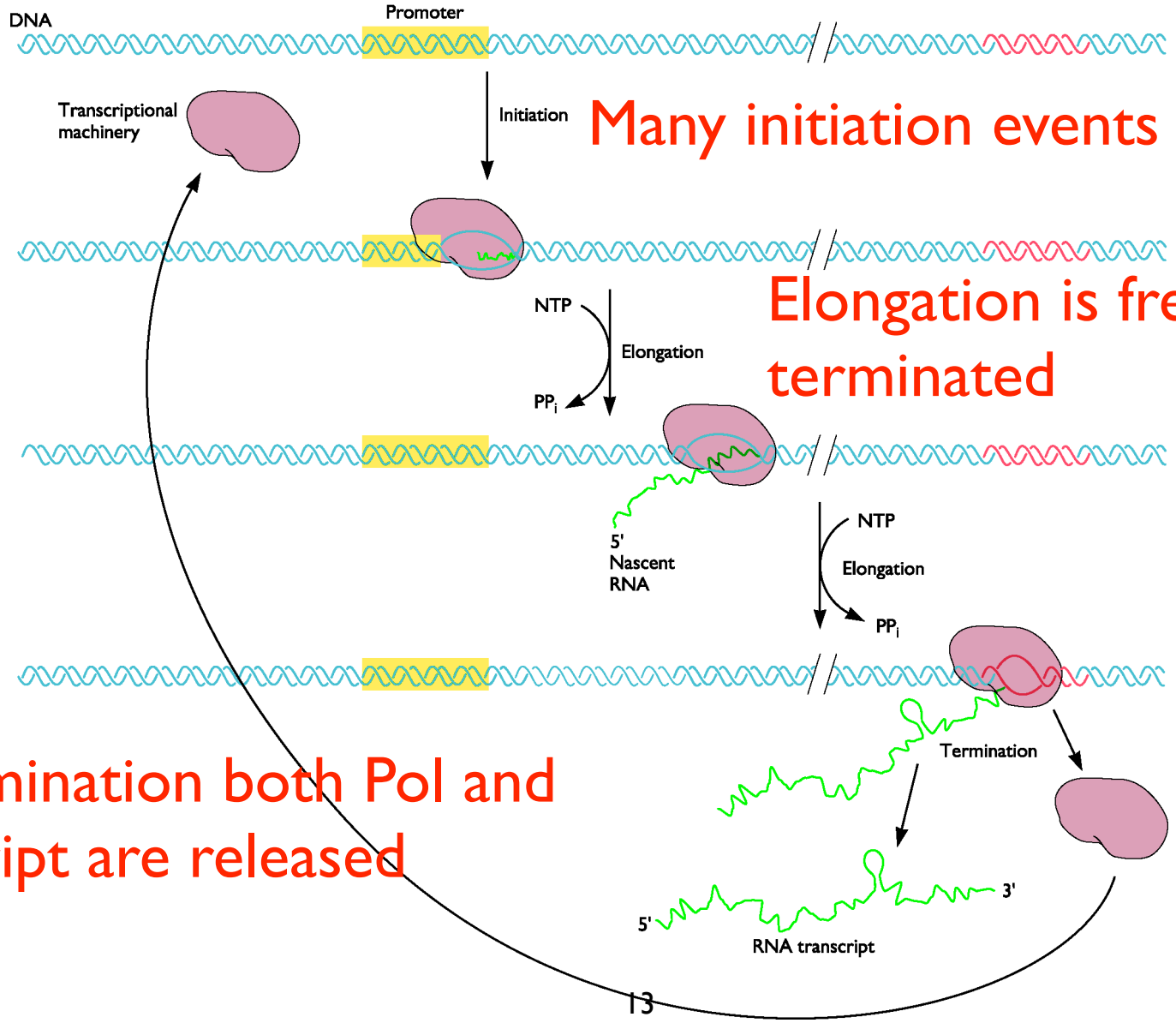
- Regulation of synthesis
- How?

  -control timing and abundance
- Why?

  - orderly synthesis allows for specific events

  -some gene products might be toxic
- What happens if things go awry?

Steps in Transcription of pre-mRNA



Many initiation events abort

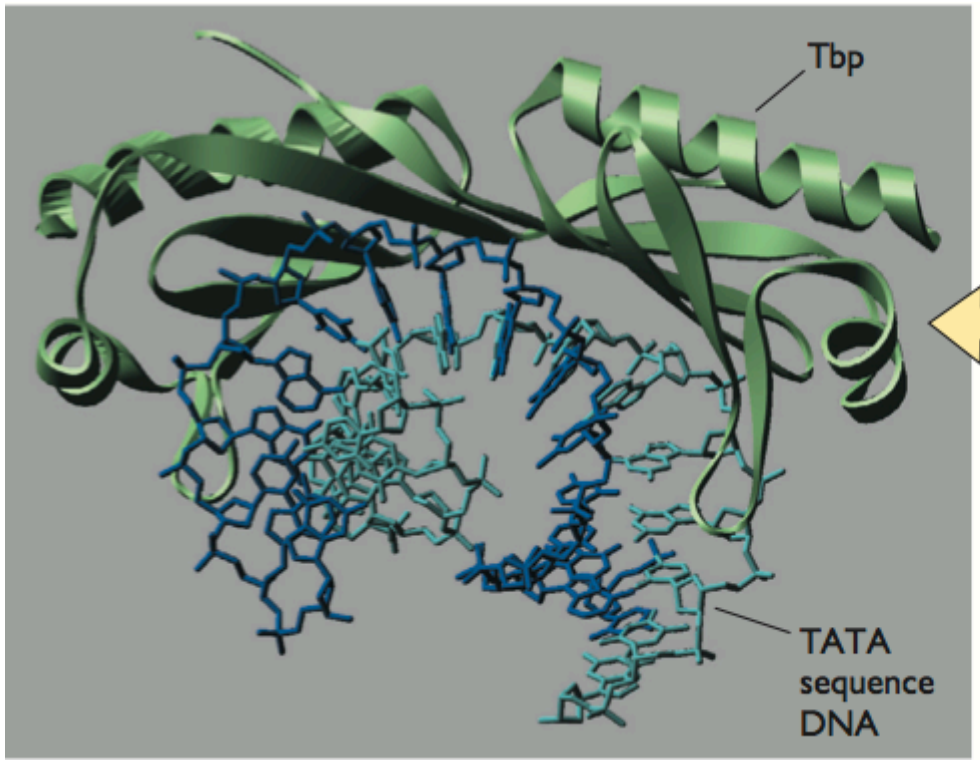
Elongation is frequently terminated

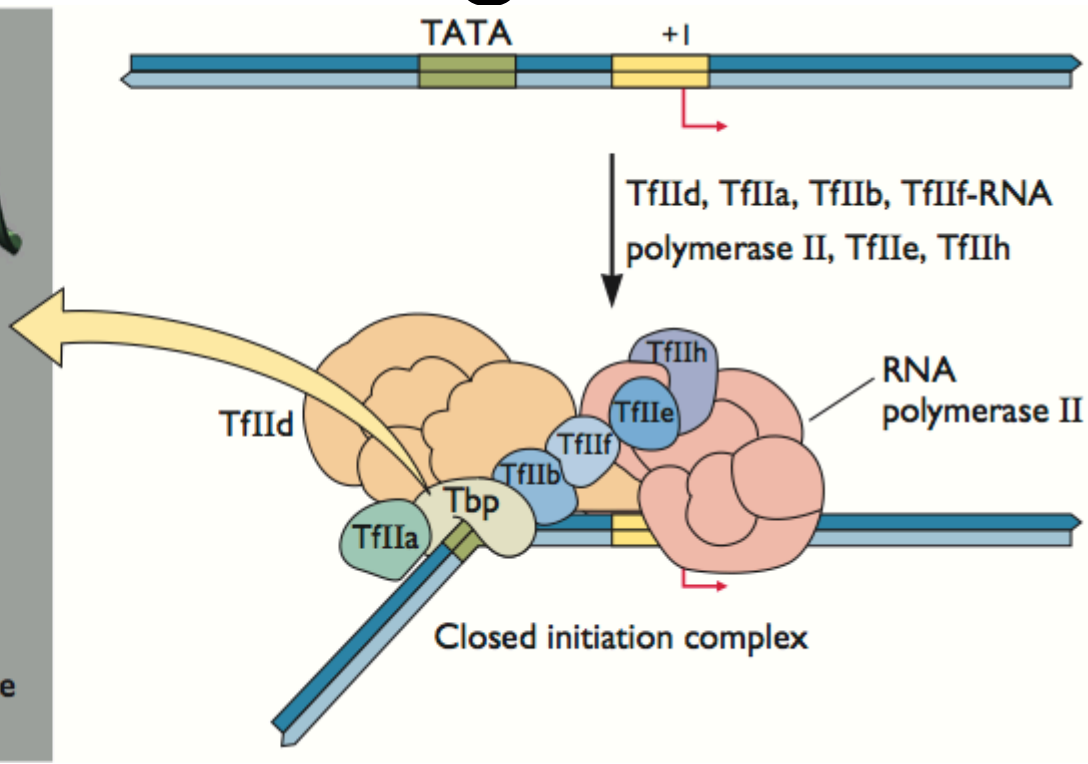
At termination both Pol and transcript are released

Steps in Initiation

- Recognition of Core Promoter
- Formation of stable closed initiation complex
- Formation of open initiation complex
- Escape from promoter
  - -regulation of Pol II by Phosphorylation
  - -promoter clearance - elongation
  - -movement of Pol complex

Order of Binding

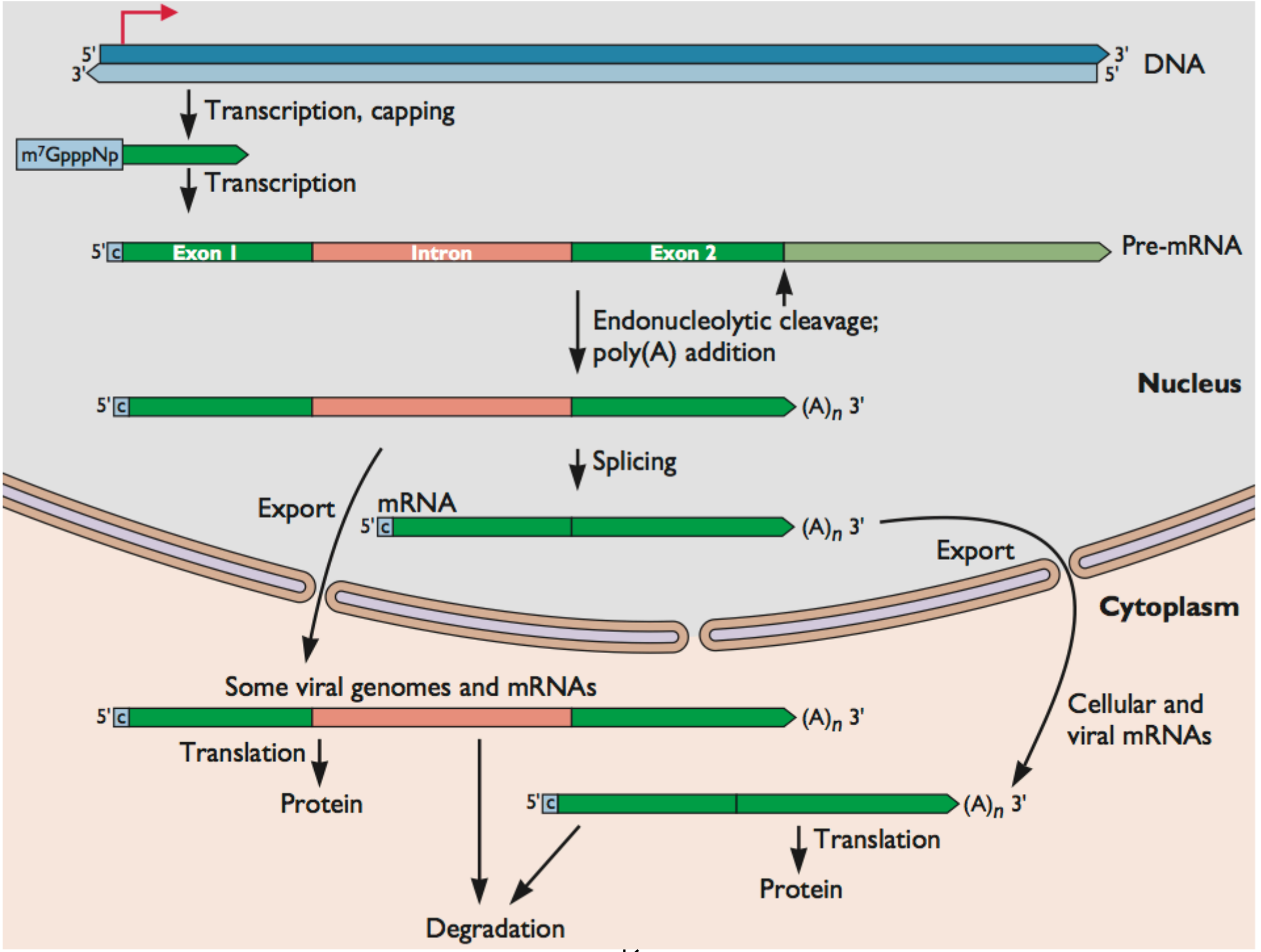




i) TfIId -Tbp & Tafs- bind & bend DNA

ii) TfIIa enters facilitating binding of Tbp to DNA

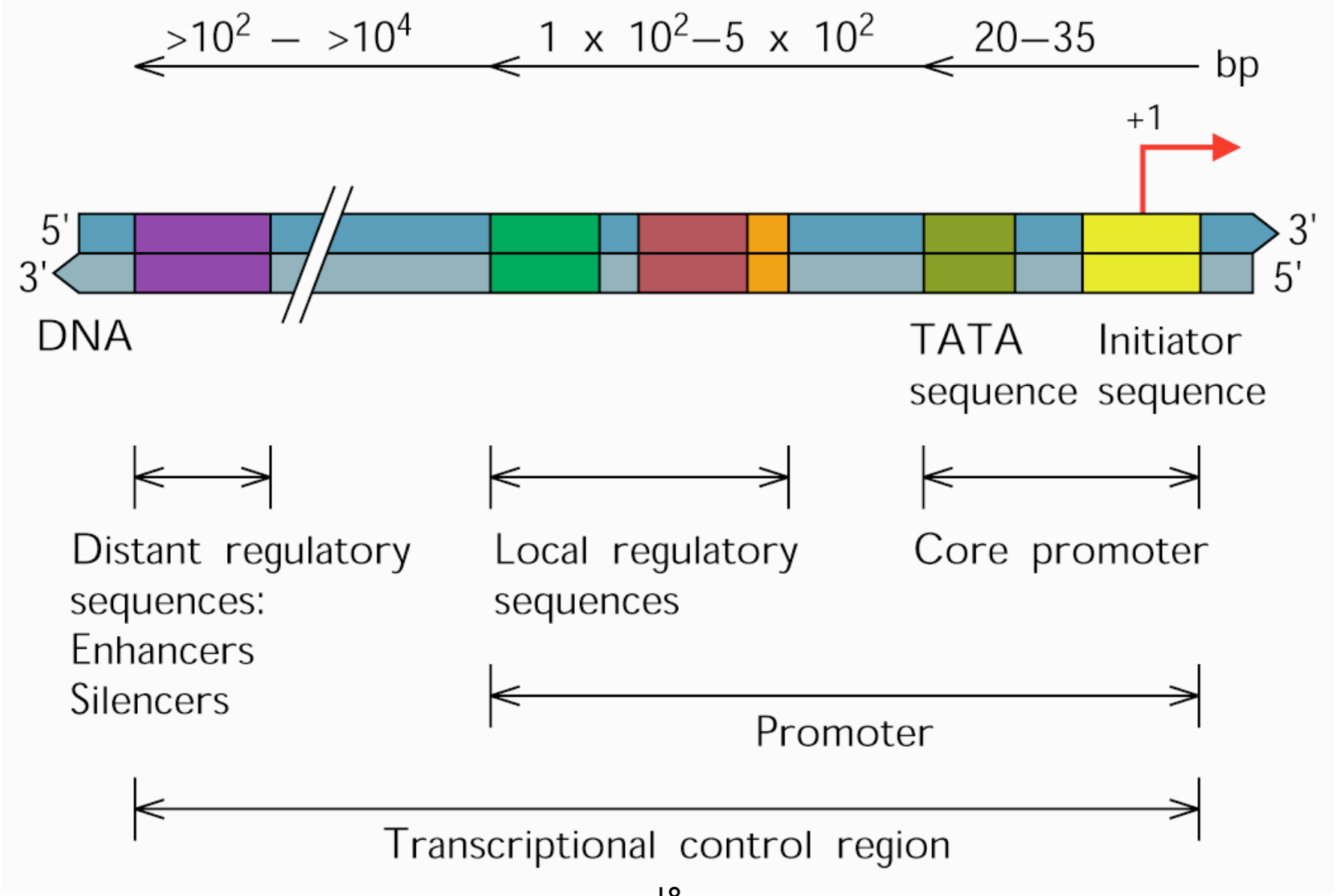
iii) Formation of closed initiation complex


5'
3'
DNA
3'
5'
Transcription, capping
m7GpppNp
Transcription
5'
c
Exon 1
Intron
Exon 2
Pre-mRNA
Endonucleolytic cleavage;
poly(A) addition
Nucleus
(A)n 3'
Splicing
Export
mRNA
Export
Cytoplasm
Some viral genomes and mRNAs
Cellular and
viral mRNAs
Translation
Protein
Translation
Protein
Degradation

Promoter Control Elements

- Core and distal elements, specific DNA sequences
- TATA - defined sequence - TFIID
- Initiator - specify accurate starts
- Distal - sites for upstream (or downstream) activator proteins
- Enhancers - position and orientation independent DNA elements
  - tissue specific or universal

Promoter Structure



Templates

- Enter the nucleus
- Templates and accessory proteins - early gene expression
- Produce a recognizable template for transcription of first wave of virus genes
- Replicate genomes to increase template # - consequences

What Does Pol II Do?

- A large complex assembly - holoenzyme
- Recognize the promoter
- Specify accurate initiation
- Responds to host and virus proteins
- Synthesize RNA transcripts

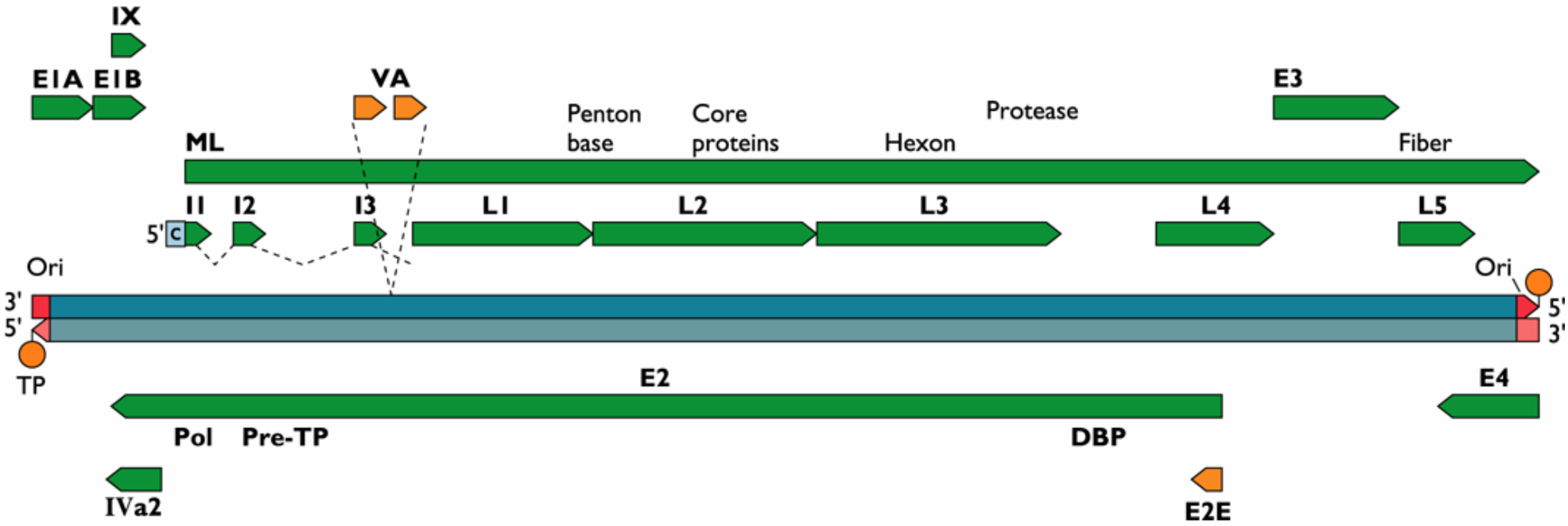
Further Steps in Regulating Transcription

- Regulation of abundance through initiation
- Availability
- Decoration of co-activators, P, Me, Ac
- Role of enhancers - change rate of initiation

Splicing

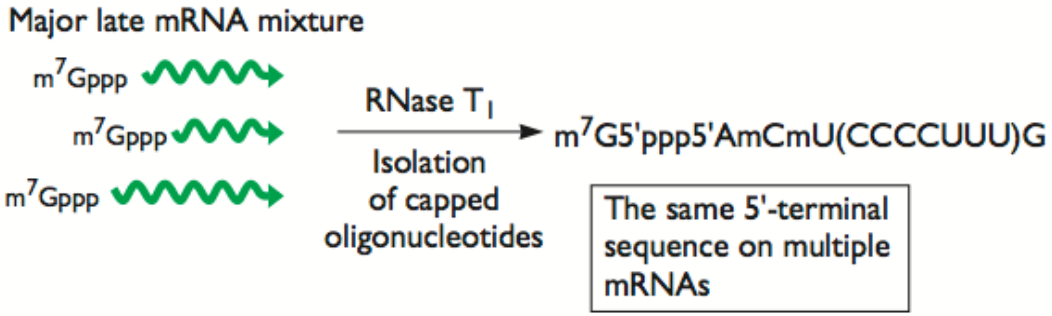
- Nuclear RNAs (hnRNAs) > mRNA
- hnRNAs have 5' caps and 3' poly A
- All Adenoviral L RNAs map to the same promoter
- Adeno L mRNAs have 4 parts, 5' terminal tripartite leader and body
- How to get small RNAs from big RNAs?

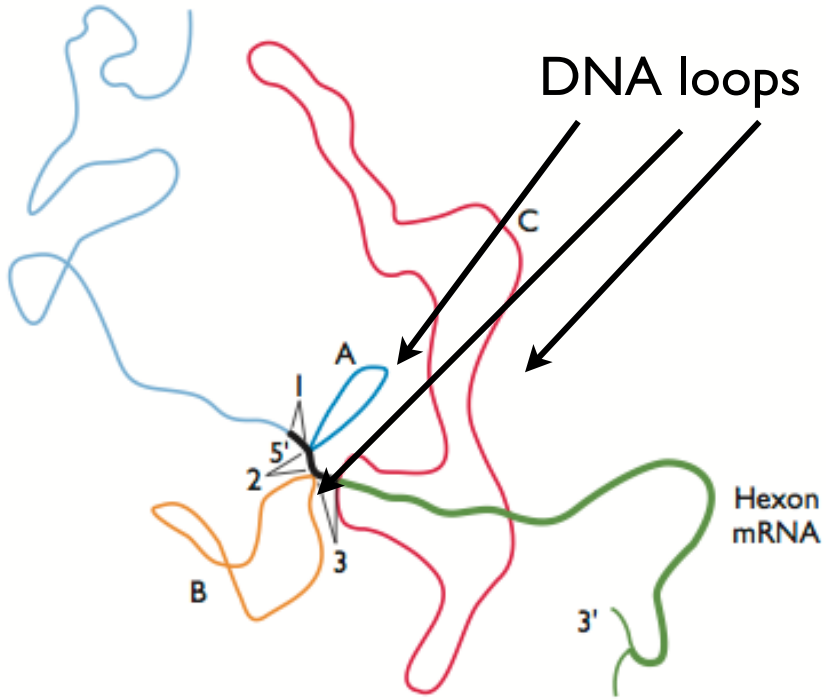
Adenovirus Transcription Map

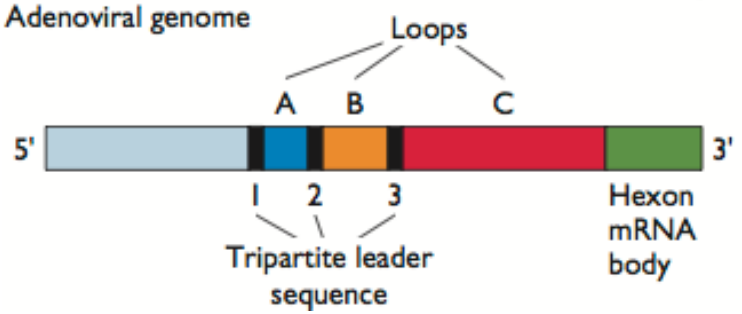


- All transcription dependent on E1A
- Late transcripts have common 5' end
- Eight transcription units, unique mRNAs

MLP-leader Sequence

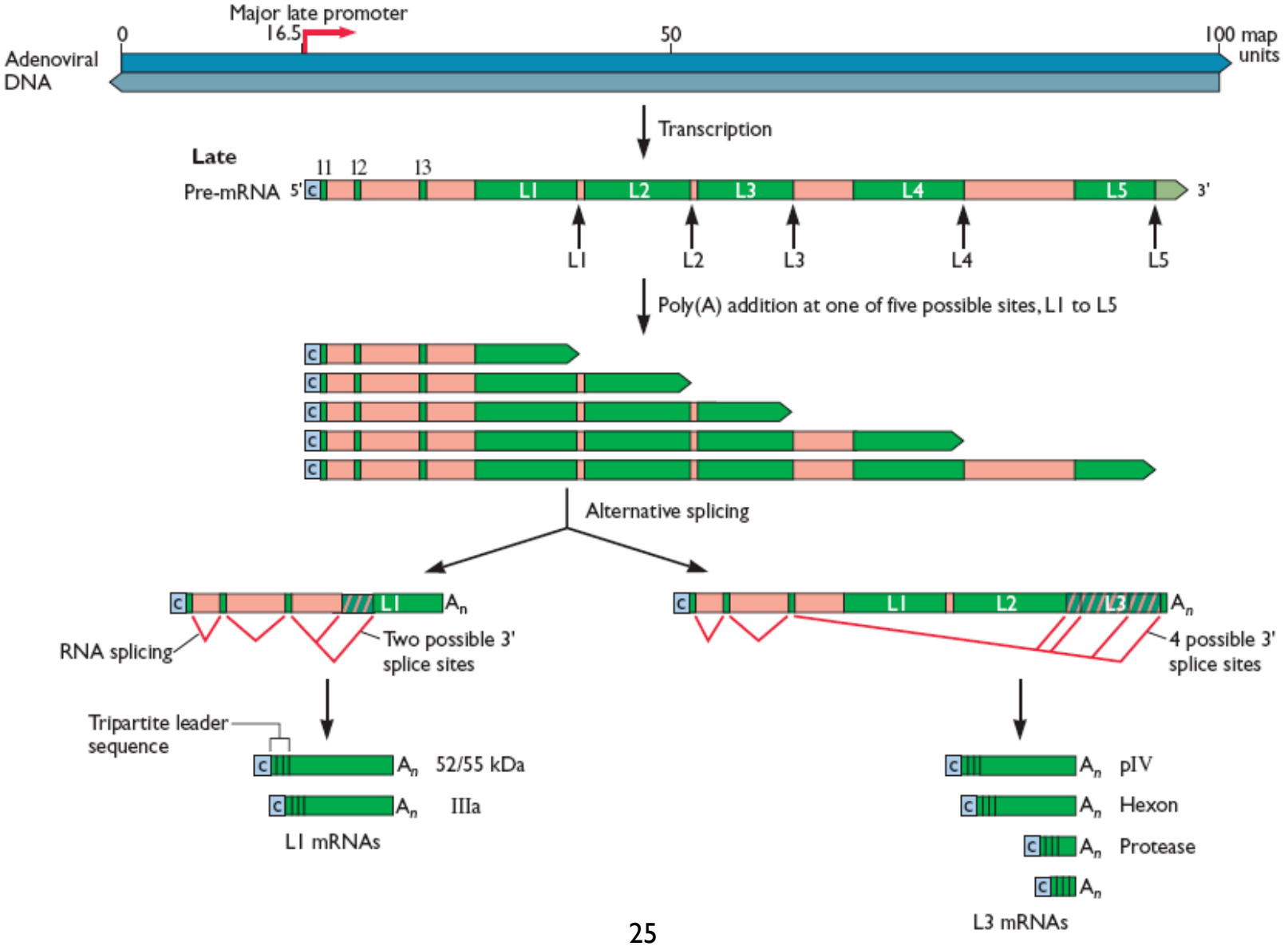




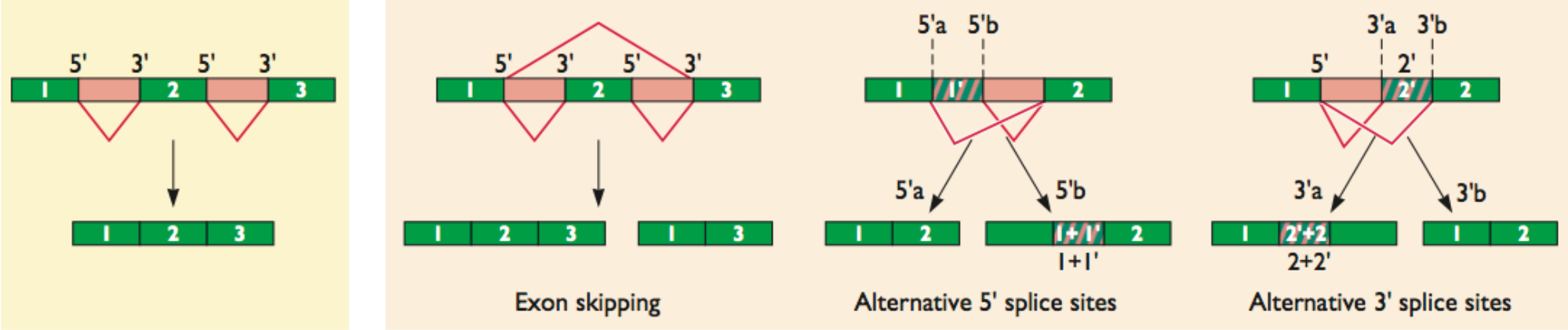


Why does the DNA loop out?

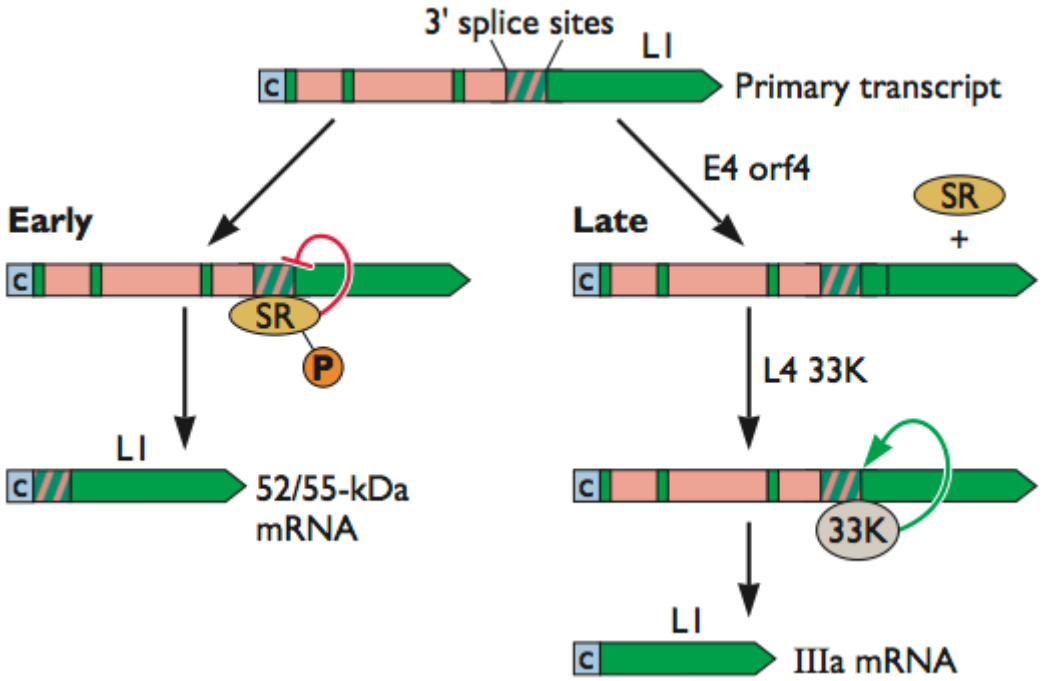
Adenovirus Alternative Splicing



Constitutive vs. Alternative Splicing



Regulation of Alternative Splicing



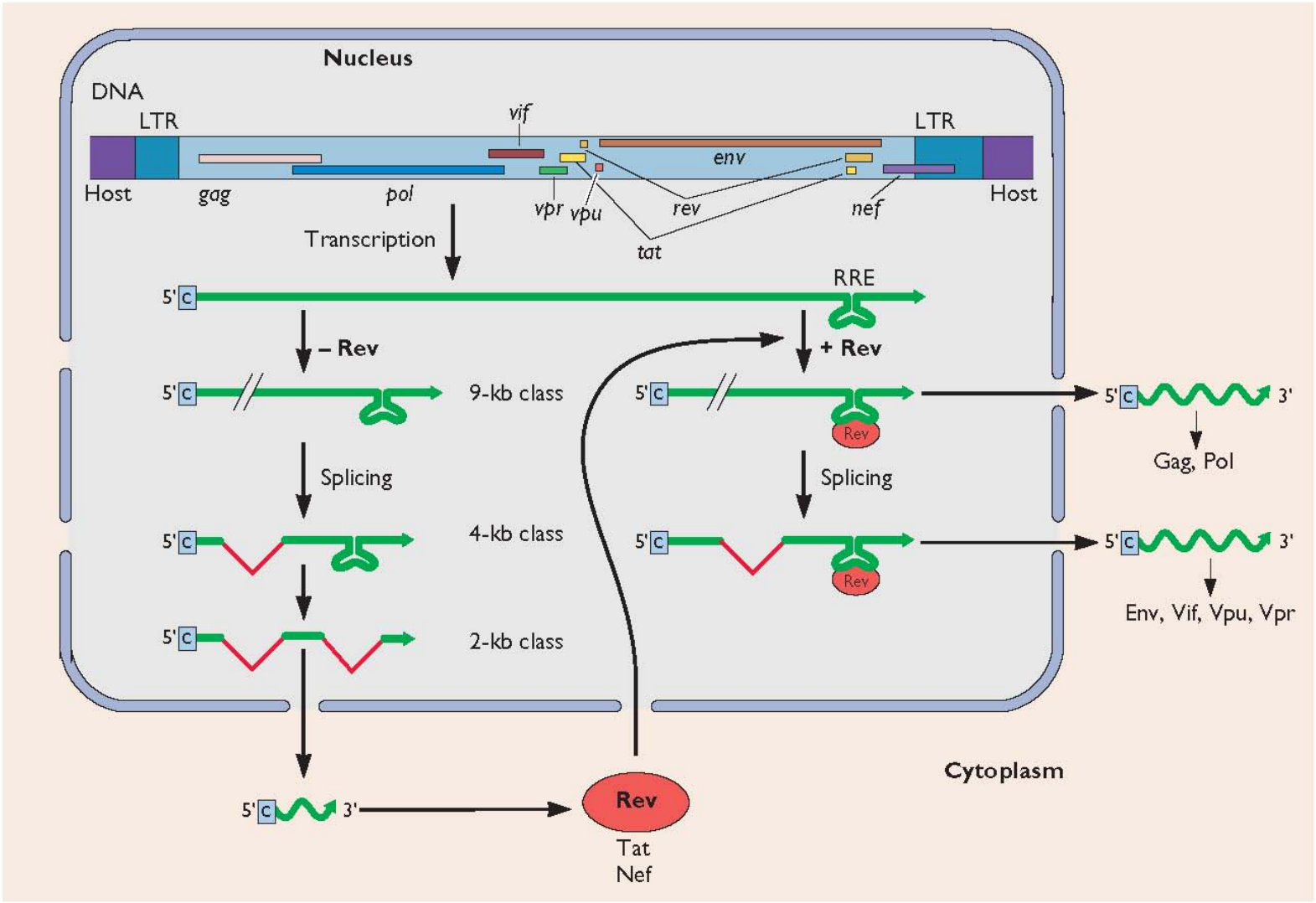
Generation of Ad IIIa Transcripts

- Early only 3' splice site for 52/55 is used
- Host SR protein blocks access to downstream site
- Ad E4 induces dephosphorylation of SR allowing, in the presence of L4, utilization of the alternative splice acceptor

Splicing = Value Added

- Introns provide numerous sites at which RNA sequences are broken and rejoined
- Splicing occurs without loss of coding information = economical
- Alternative splicing creates new functional genes
- Coding information of a small DNA genome is expanded

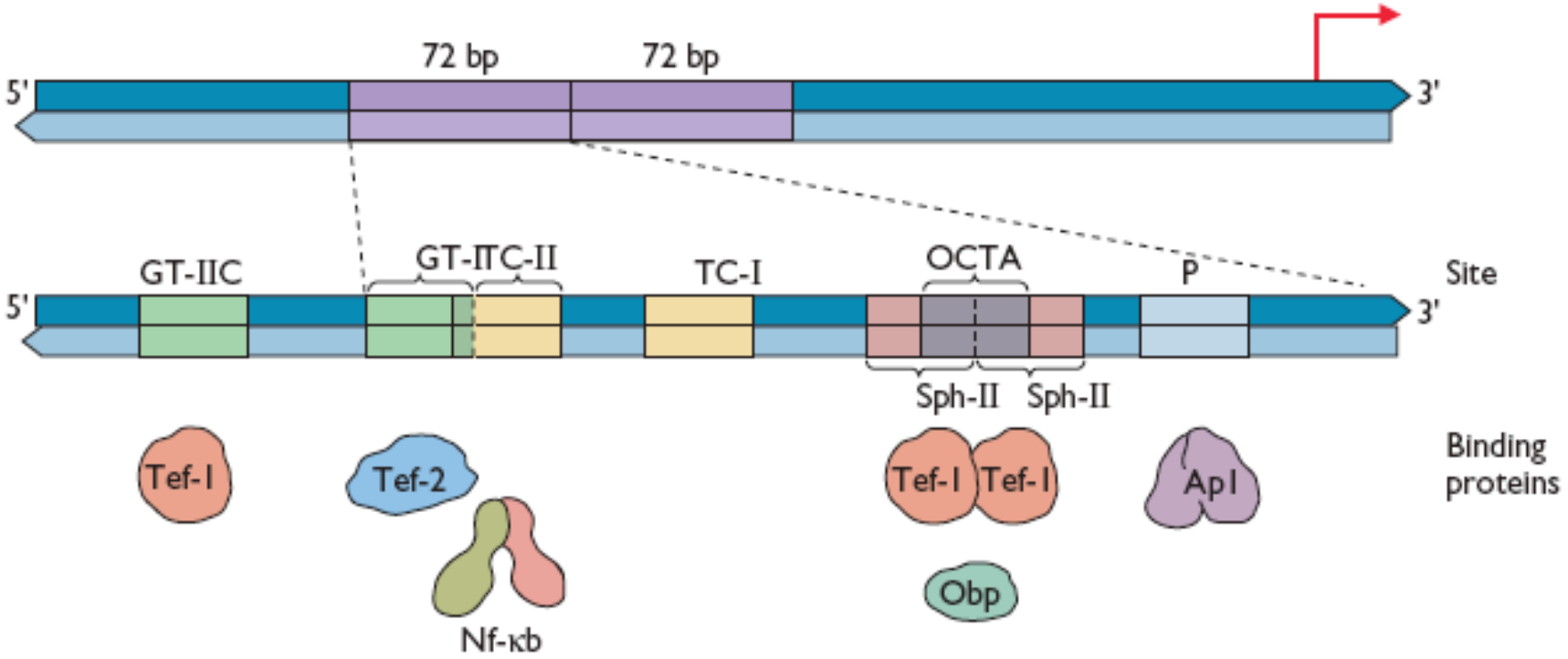
Rev PromotesHIV Alternative Splicing



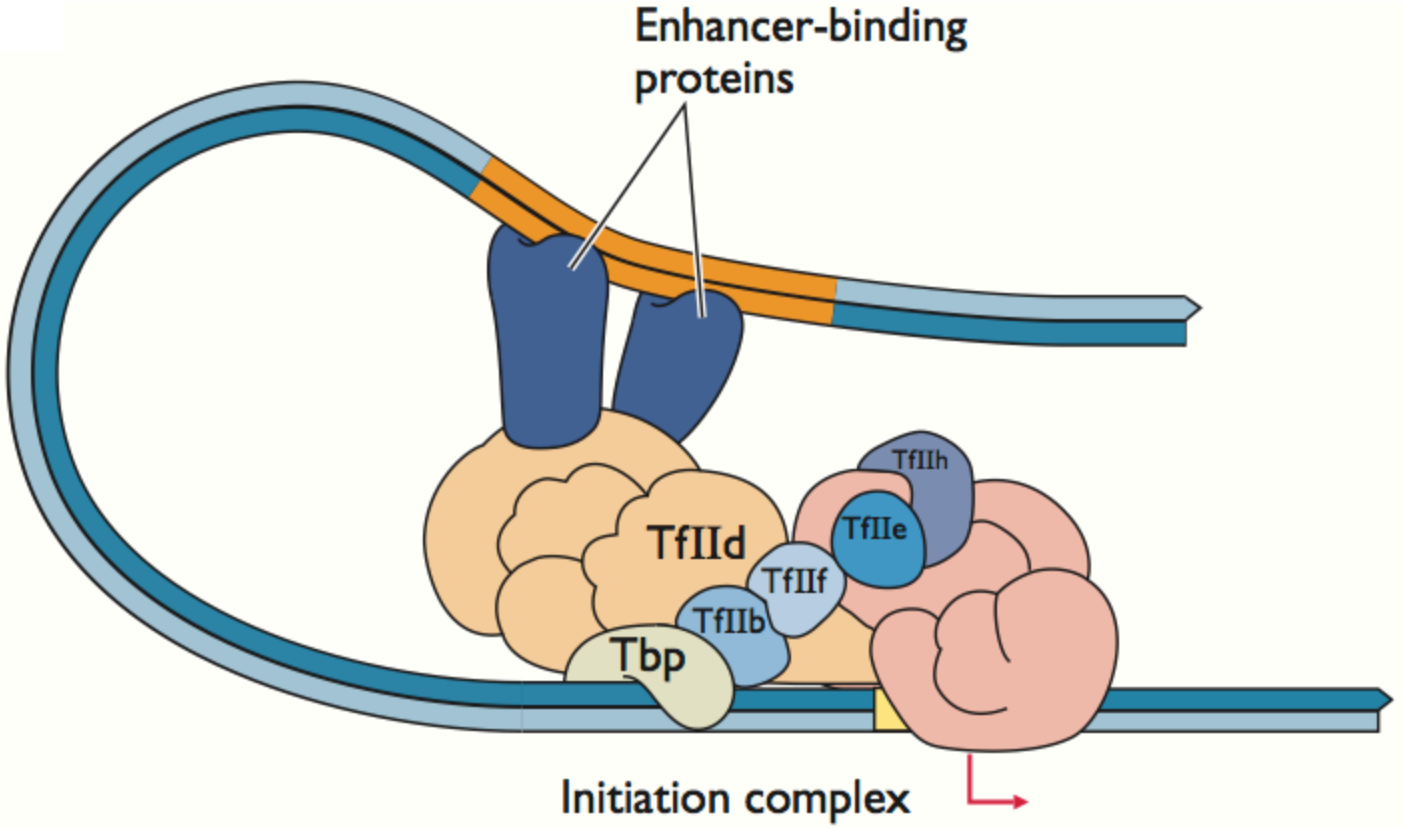
Enhancers

- Work at a distance
- Orientation independent
- Can work in trans
- Enhance initiation

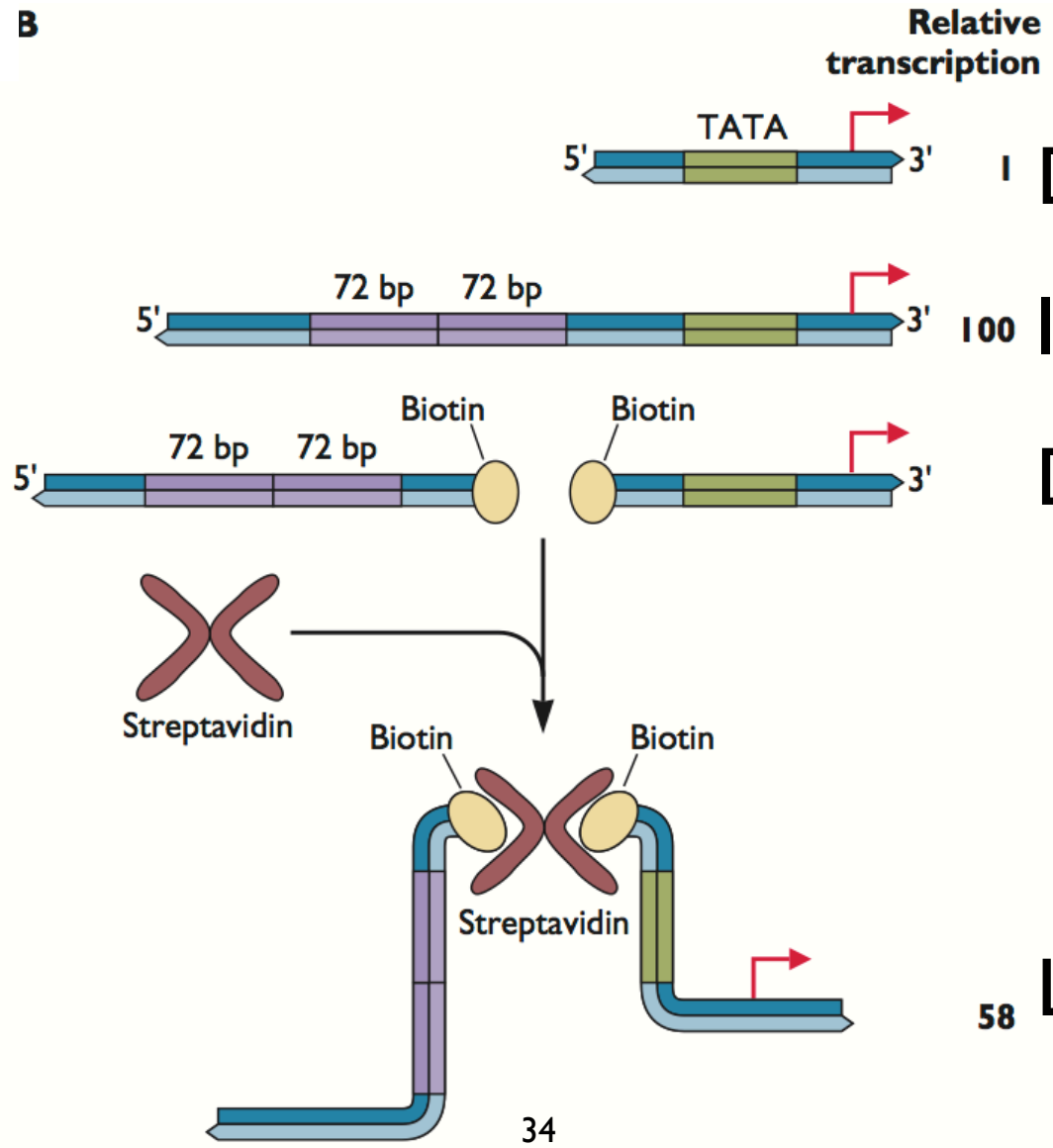
Enhancer Structure



How do Enhancers Work?



Enhancers Work in Trans

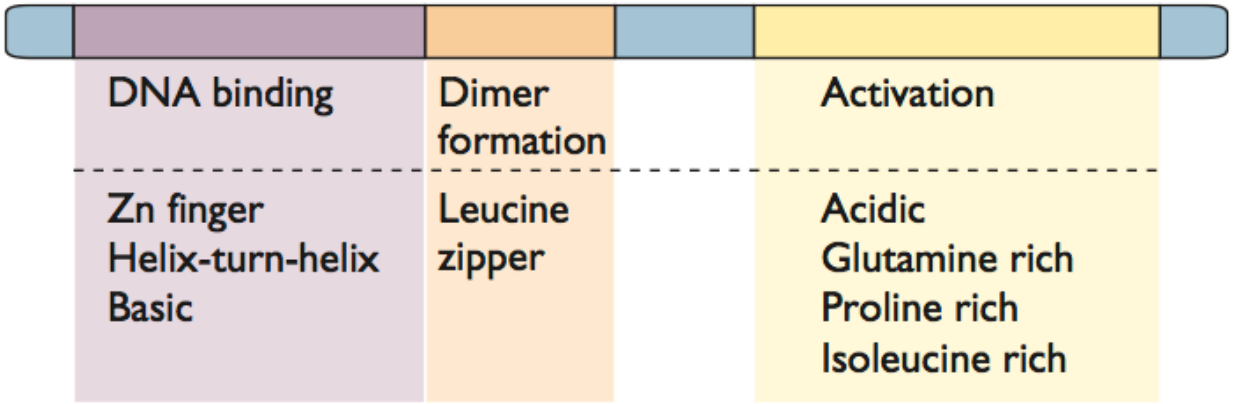


Regulation of and by Host Proteins

- Viruses use host and/or virus-specified proteins to regulate gene expression
- They either encode and/or bring with them co-activating molecules
- Cell type specificity can limit expression
  - co-activator molecules can be organ or species specific

Regulatory Protein Domains

- Regulatory molecules are composed of multiple domains that contribute to virus gene regulation
- DNA binding
- Activator/Repressor
- Interactor
- Multimerization



Viral Transcriptional Activators

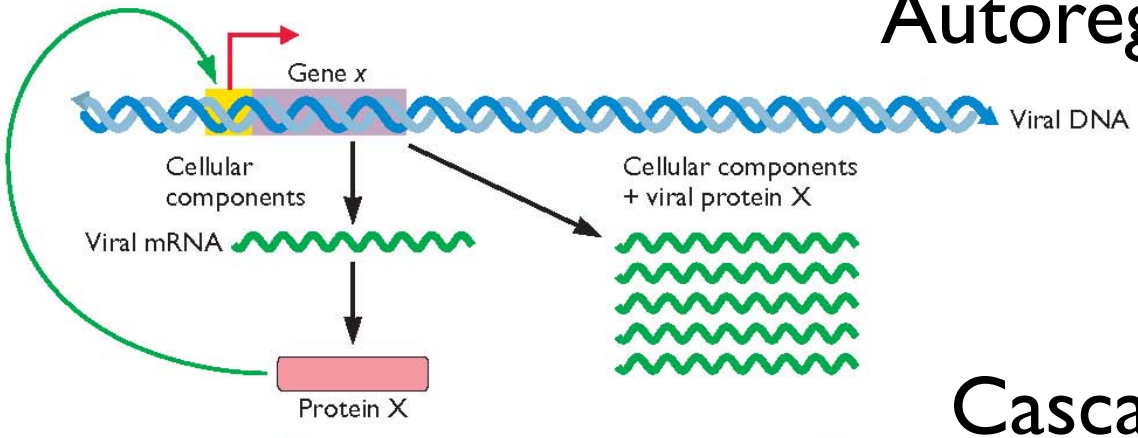
- Autoregulatory molecules
  -SV40 Tag, HSV ICP4
- Some bind DNA - T, EBNA, ICP4, E2
- Some bind host proteins - HSV VP16
- Others liberate host TA's - T, E1A, E7

Patterns of Regulation

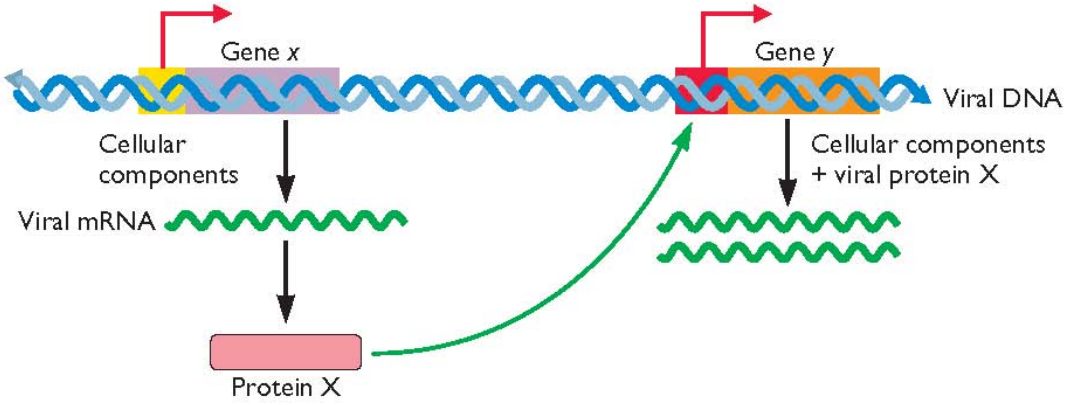
- Proteins interact with Pol II to establish regulatory circuits
- Positive Autoregulatory Loops
  - -alter the rate of transcription initiation
  - -virus proteins stimulate transcription
- Negative Autoregulatory Loops
  - -repress gene expression
- Transcriptional Cascade
  - -transcriptional units are activated in a fixed sequence

Regulatory Machines

Positive vs. Negative Autoregulatory Loops



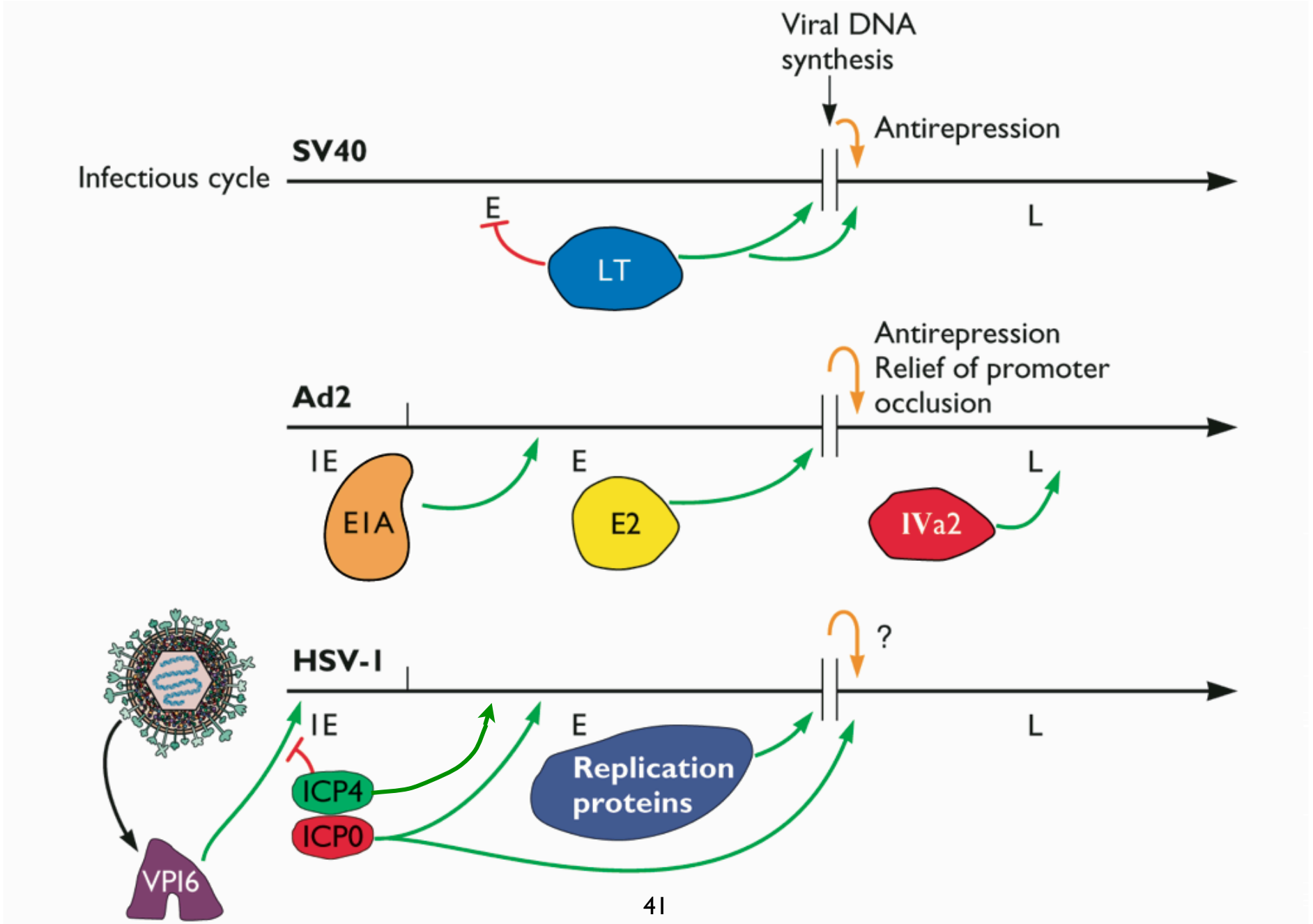
Cascade Regulation



Transcriptional Cascade

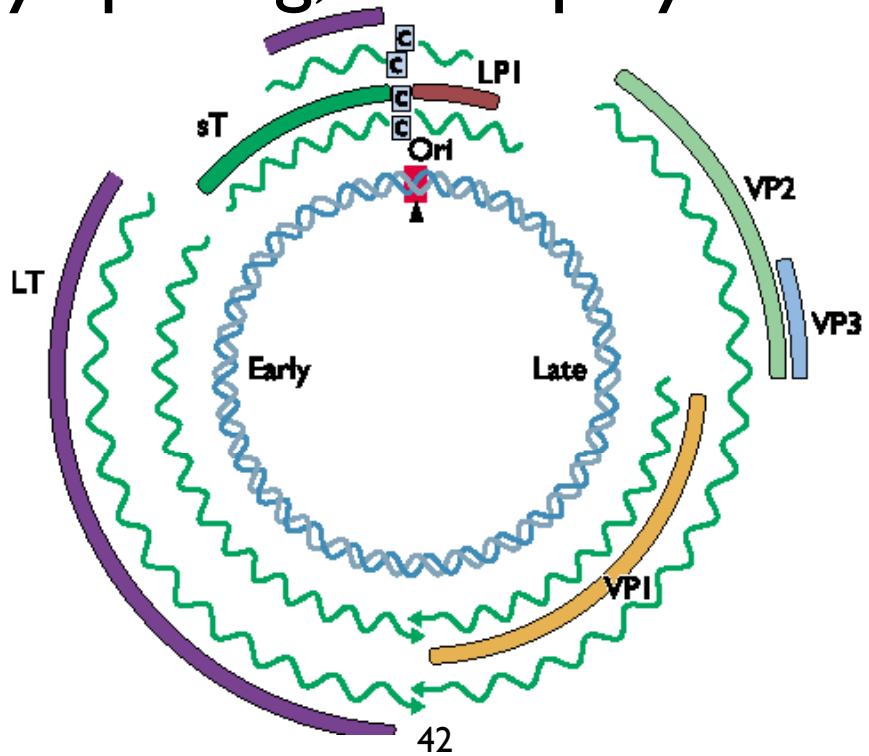
- Transcription of viral genes in a temporally controlled sequence
- Immediate early and early proteins
- Transcription of late genes
- Ensures coordinated production of DNA genomes and structural proteins, frees template from repressors
- Activating proteins can induce transcription of host and viral genes and repress transcription of their own genes

Transcription Made Easy



Polyomavirus Transcription

- E and L units transcribed from a common region, no nucleosomes
- E and L transcripts contain overlapping mRNAs, regulated by splicing, share poly A sites



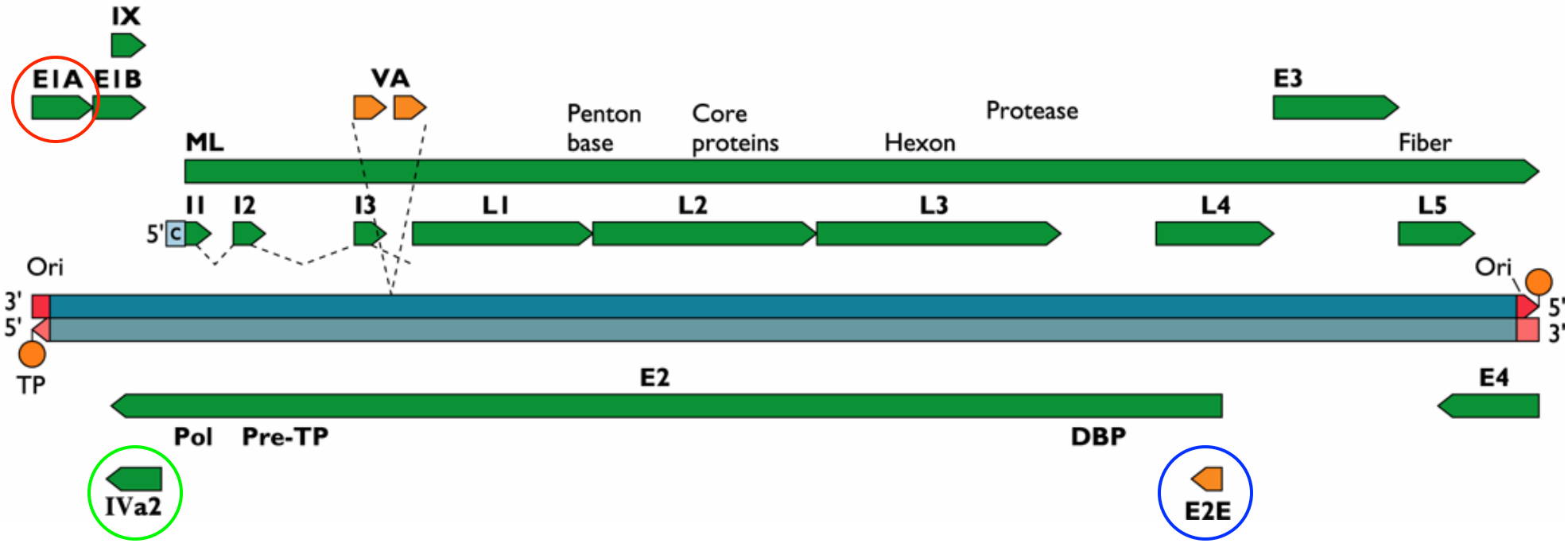
How Does T Work?

- T binds polyomaviridae Oris as a hexamer
- Early promoter dampened
- Late promoter activated
- Early transcripts are decreased relative to Late

Adenovirus Transcriptional Regulation

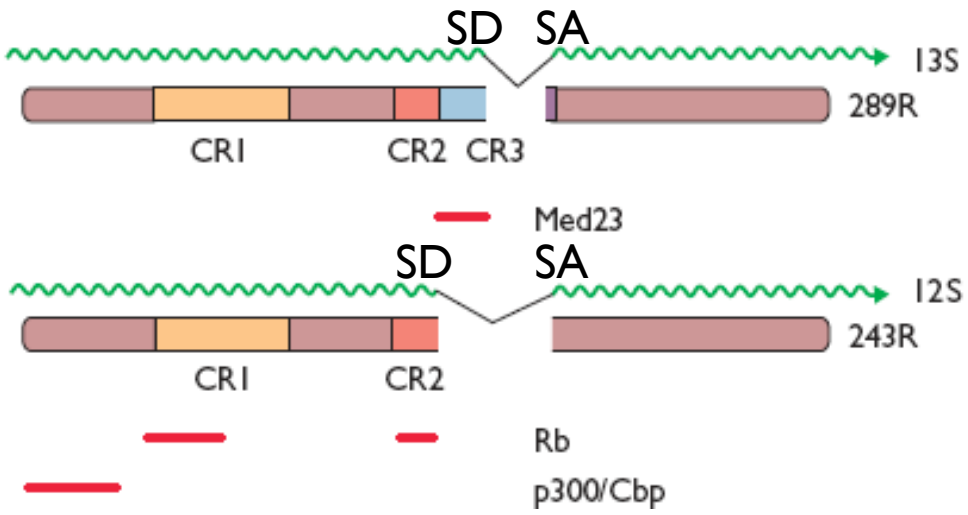
- Three virus proteins and DNA synthesis govern phase transitions
- E1A, necessary for transcription of all E transcription units
- E2 required for DNA synthesis and entry into L transcription phase
  -increases initiation from MLP
- IVa2 enhances L gene transcription

Adenovirus Transcription Units



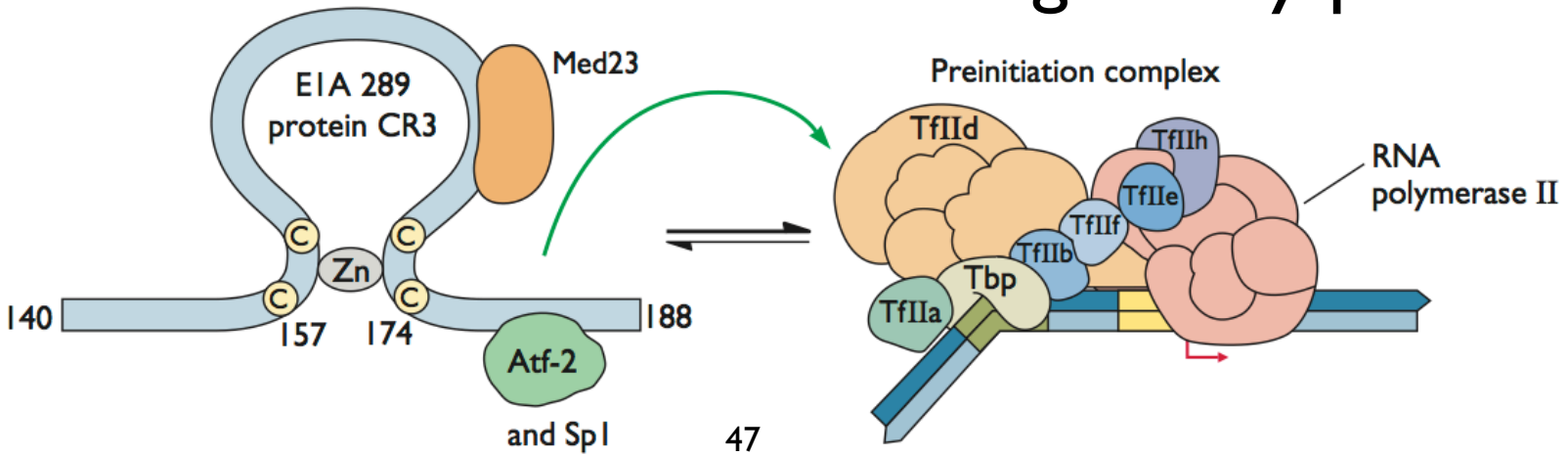
E1A Gene Transcript Family

- Differential splicing results in two proteins of 243 and 289 amino acids with a conserved reading frame
- CR3 stimulates early gene transcription

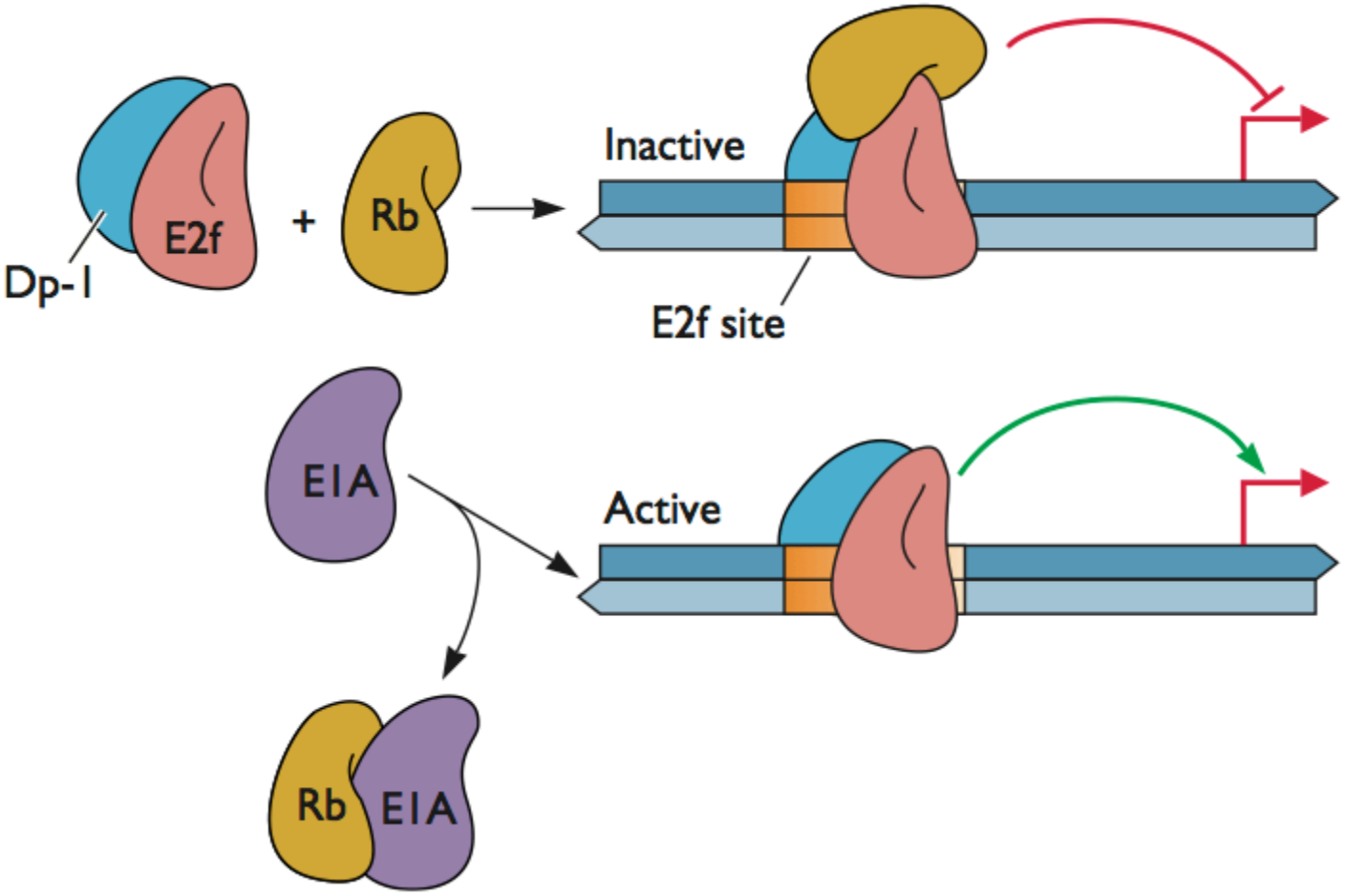


How Does E1A Work?

- E1A does not bind DNA
- E1A does bind , Atf-2, Sp1 and Med23
  -binding to Med23 stimulates assembly of preinitiation complexes
- Also activates by another mechanism
  -interaction with host regulatory proteins



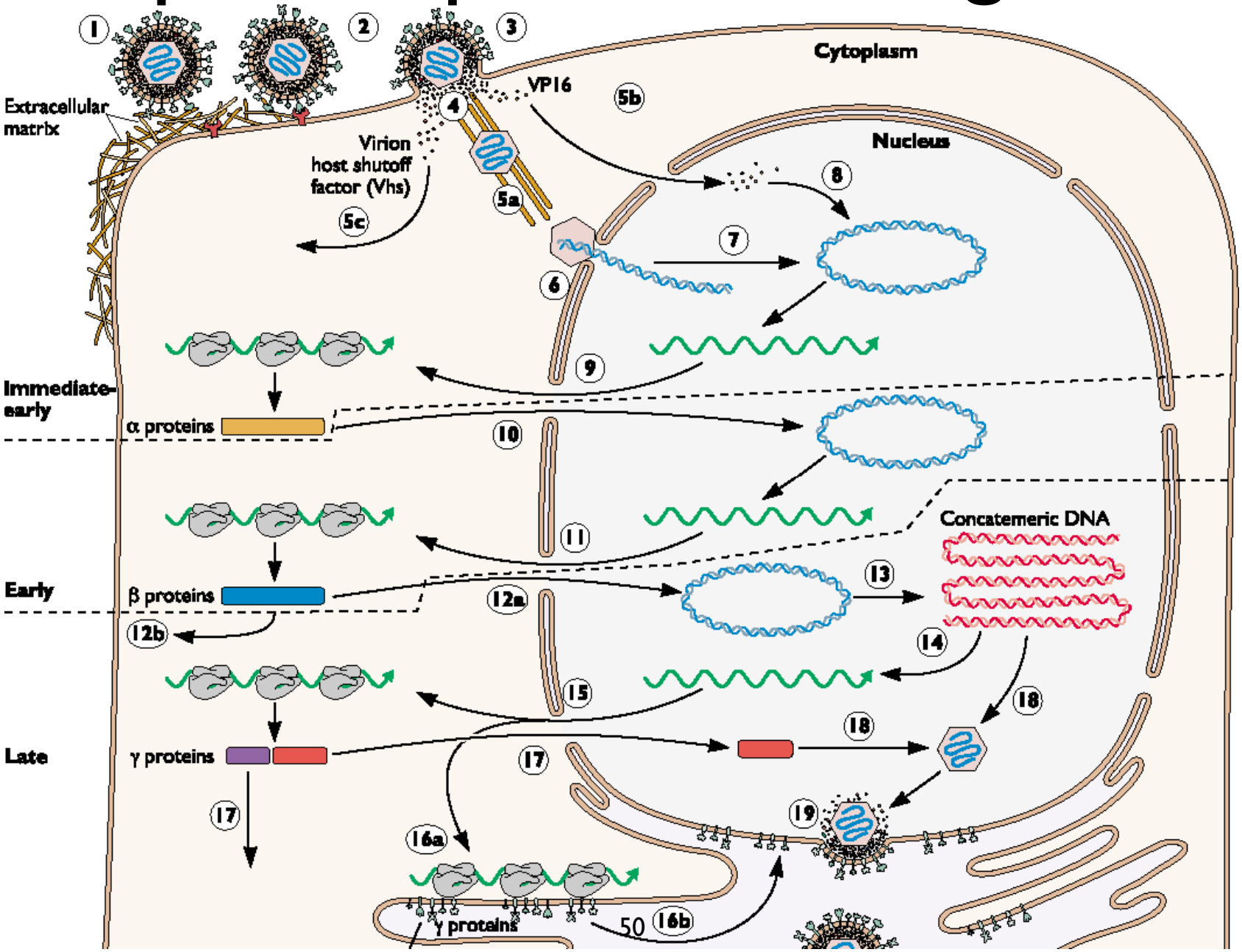
Interaction of E1A with Rb



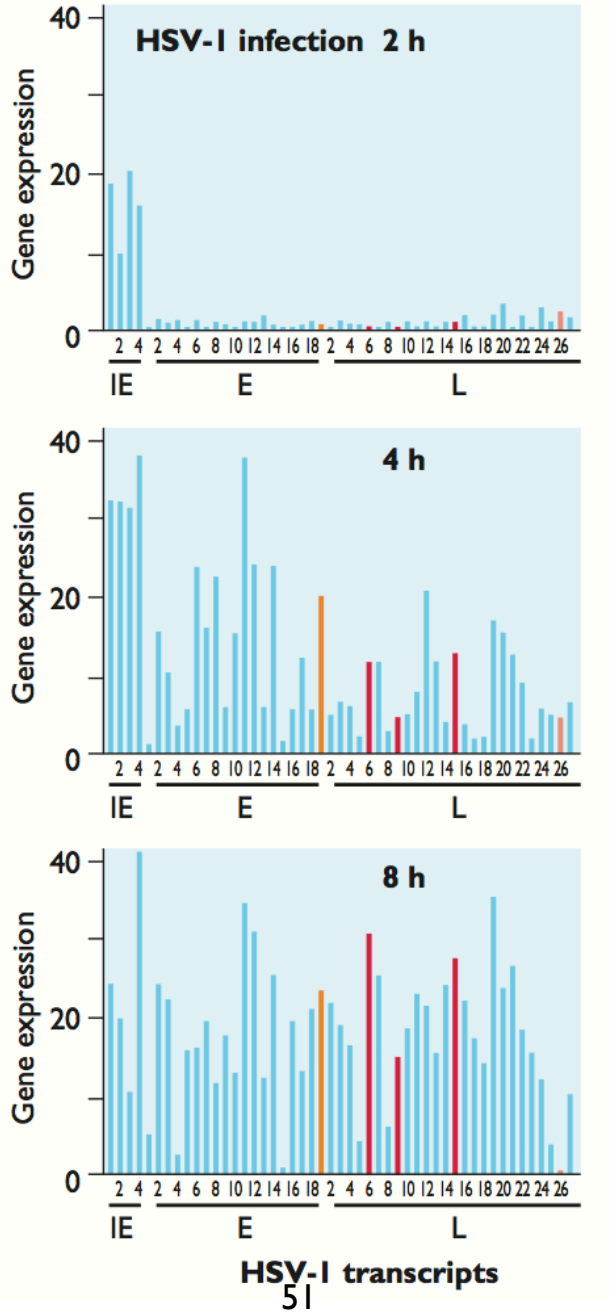
Herpesvirus Regulatory Cascade

- Initiated by VP16, a virion associated protein
- Activates IE transcription
- IE proteins control transcription from all virus genes
- Expression of E genes and DNA synthesis
- Expression of DL and L gene, DNA dependency
- Packaging of VP16 into new virions
- Coordinate regulation in a temporal fashion

Herpes Simplex Gene Regulation



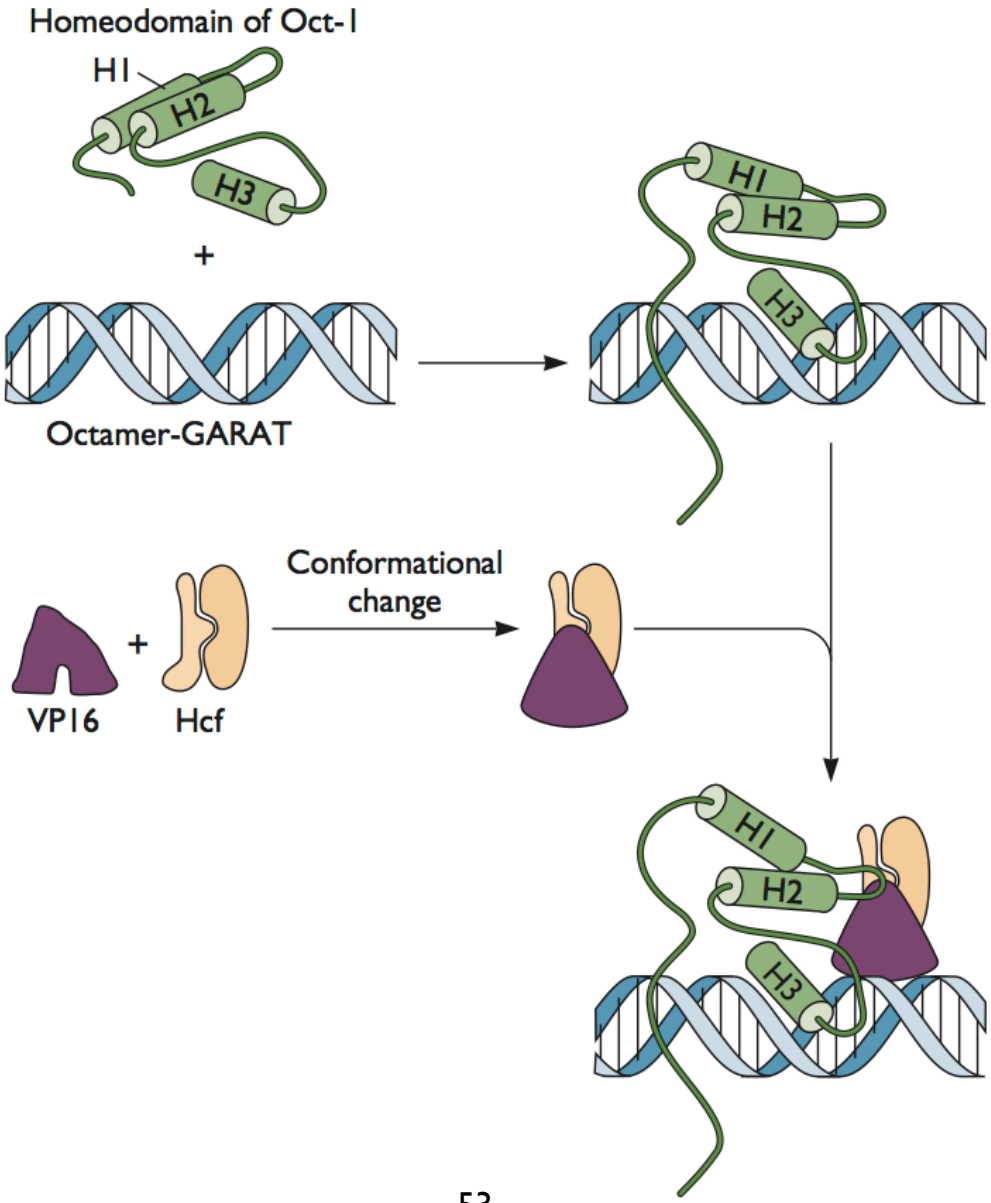
Distinct Patterns of Accumulation of HSV RNA



VP16

- Potent C-terminal acidic activator
- Does not bind DNA directly
  -but requires a TAATGARAT motif in virus promoters
- Associates with HCF and Oct-1
  -they provide promoter specificity
- Stimulates initiation and elongation of transcription
- Specific for IE promoters

Interactions by VP16



Export

- A primary transcript does not become a mRNA until it is exported
- Export is usually accomplished by host proteins and the transcript uses nuclear pores to exit
- A protein complex that marks mature RNAs for export from the nucleus is assembled during splicing
- Exportins shuttle between the nucleus and cytoplasm carrying RNA as their cargo

Today's Concepts

- Transcription is complicated
- Control is at many levels
- Host and viral proteins regulate transcription
- Viral gene expression is coordinately regulated in a temporal manner