Transcription and RNA processing

Lecture 7
Biology 4310
Virology
Spring 2021

None loves the messenger who brings bad news
—Sophocles
Influenza virus
Reovirus
Rotavirus
Hepatitis B virus
Parvovirus
Retrovirus
Adenovirus
Herpes simplex virus
Polyoma- and Papillomaviruses
Poliovirus
Reovirus
Rotavirus
Influenza virus

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

A. Hepadnavirus

B. Parovirus

C. Retrovirus

Which viral genomes do not need conversion?
# Eukaryotic DNA-dependent RNA polymerases

*All initiate RNA synthesis de novo (no primer needed)*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cellular RNA</th>
<th>Viral RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA pol I</td>
<td>Pre-rRNA</td>
<td>None known</td>
</tr>
<tr>
<td>RNA pol II</td>
<td>Pre-mRNA&lt;br&gt;Pri-miRNA&lt;br&gt;SnRNA&lt;br&gt;LncRNA</td>
<td>Pre-mRNA&lt;br&gt;Pri-miRNA&lt;br&gt;HDV genome RNA and mRNA&lt;br&gt;HHV8 PAN RNA</td>
</tr>
<tr>
<td>RNA pol III</td>
<td>Pre-tRNAs&lt;br&gt;5S rRNA&lt;br&gt;U6 snRNA</td>
<td>Ad-2 VA RNAs&lt;br&gt;HBoV1 Boca SR&lt;br&gt;MHV68 pri-miRNA</td>
</tr>
</tbody>
</table>

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

- >10^2 – >10^4 base pairs
- 1 x 10^2 – 5 x 10^2 base pairs
- 20 – 35 base pairs

DNA

Binds TFIID

TATA sequence

Initiator sequence

Promoter

Transcriptional control region

(Specific DNA sequences that bind proteins)

Distant regulatory sequences:
- Enhancers
- Silencers

Local regulatory sequences

Core promoter

Specifies accurate starts

(Position and orientation independent)
Regulatory sequences in transcriptional control regions

Ad2 major late

Cpf
Usf1

SV40 early

Spl

Spl

Spl

Spl

Spl

Spl

Ad2 E2 early

Atf
E2f

E2f

bp -100

+1
Initiation of transcription by RNA polII
Enhancer-binding proteins up to 10,000 bp away!

Initiation complex

Enhances initiation!
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

DNA binding: Zn finger, Helix-turn-helix, Basic
Dimer formation: Leucine zipper
NLS: Acidic
Activation: Glutamine rich, Proline rich, Isoleucine rich
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

<table>
<thead>
<tr>
<th>Origin of transcriptional components</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host only</td>
<td>Retroviruses with simple genomes</td>
</tr>
<tr>
<td></td>
<td>Caulimoviruses</td>
</tr>
<tr>
<td></td>
<td>Circoviruses</td>
</tr>
<tr>
<td>Host plus one viral protein</td>
<td></td>
</tr>
<tr>
<td>Viral protein transcribes late genes</td>
<td>Bacteriophages T3, T7</td>
</tr>
<tr>
<td>Viral protein regulates transcription</td>
<td>Hepadnaviruses, paroviruses, papillomaviruses, polyomaviruses,</td>
</tr>
<tr>
<td></td>
<td>retroviruses with complex genomes</td>
</tr>
<tr>
<td>Host plus &gt;1 viral protein that stimulate transcription</td>
<td>Adenoviruses, herpesviruses</td>
</tr>
<tr>
<td>Viral</td>
<td>Mimiviruses, Poxviruses</td>
</tr>
</tbody>
</table>

**Recognition of viral promoters!**
Regulation of transcription by viral proteins

Cascade regulation

Positive autoregulatory loop
(may also be negative)
Viral transcriptional programs: SV40

Time after infection

SV40

Viral DNA synthesis

Antirepression

E

LT

Ori

Early

Late

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Regulation of SV40 late promoter by cellular repressors

ibp = initiator binding protein, a cell protein

- Early phase
- Late phase

Graph showing relative concentration of viral genomes, viral late RNA, and initiator binding protein over time after infection.
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
Go to:

b.socrative.com/login/student
room number: virus

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
Modification of mRNA: Cleavage and polyadenylation

- Transcription, capping
- Endonucleolytic cleavage; poly(A) addition
- Splicing
- Export
- Translation
- Degradation
- ATP
- Poly(A) addition site
- CPSF
- CFIm
- CSTF
- Poly(A) polymerase
- Cleavage
- Slow polyadenylation
- Degradation
- PABP
- ATP
- PABP
- Rapid polyadenylation

~200 A

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## Addition of poly(A) to viral mRNAs

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

A  Constitutive splicing

B  Alternative splicing

Exon skipping

Alternative 5' splice sites

Alternative 3' splice sites
Viral proteins can regulate alternative splicing.
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
Export of unspoiled 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
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

IncRNAs

circRNAs
Synthesis and function of miRNAs

pri-miRNA → pre-miRNA → Mature miRNA

DROSHA/DGCR8 cleavage

Passenger strand removal and GW182 association

GW182

Mature miRNA

AGO

AGO loading

Dicer

TRBP

Dicer processing

Cytoplasm

Nucleus

mRNA targeting

Transitional suppression Deadenylation

AGO2 cleavage mRNA degradation

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Liver-specific miR-122 promotes hepatitis C virus replication
Polyomavirus miRNA may promote persistence

SV40

Pre-miRNA

3' 5' miRNA

3' 5' Early mRNA

3' 5'

Cleavage

3' 5'
Effect of IncRNAs on virus reproduction

Cytoplasm

SV

VSV

ACOD1

Acidified endosome

GOT2

Reshaping of the metabolic environment

Increased replication of:
SV
VSV

Nucleus

Transcription
HIV-1 proviral DNA

NRAV

ZONAB

IFIT2
IFIT3
IFITM3
MxA

Increased replication of:
IAV

HsST

WDR5

IFNγ

Increased persistence of TMEV

JncRNA#32

ATF2

CCL5
IRF7

Reduced replication of:
EMCV
HCV
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)

Paroviridae (4–6 kb)

Adenoviridae (36–48 kbp)

Herpesviridae (120–220 kbp)

Poxviridae (130–375 kbp)

Terminal loop

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Next time: Viral DNA replication