RNA directed RNA synthesis

Lecture 6
Biology 3310/4310
Virology
Spring 2019

Truth is ever to be found in the simplicity, and not in the multiplicity and confusion of things
-- Sir Isaac Newton
Some RNA history

- 1935 - Stanley crystallizes TMV
- 1936 - TMV crystals contain 5% RNA
- 1944 - DNA is genetic material
- 1952 - Hershey-Chase experiment
- 1953 - Structure of DNA
- 1956 - TMV nucleic acid is infectious; first demonstration that RNA can be genetic material
- By 1959, RNA was identified in many animal viruses
- 1960s - studies on viral RNA replication begin
Influenza virus
VSV
Reovirus
Rotavirus
Hepatitis B virus
Parvovirus
Retrovirus
Adenovirus
Herpes simplex virus
Poliovirus
VII

Retrovirus
- RNA
Poliovirus
+ RNA → - RNA
Parvovirus
+ DNA
- DNA
± DNA
± DNA
+ mRNA
Influenza virus
VSV
Reovirus
Rotavirus
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Identification of RNA polymerases

RNA polymerase activity in infected cells

Cell extract

ATP, UTP, GTP, CTP

Incubate

Measure RNA synthesis

RNA polymerase activity, cpm/mg protein

Hours post-infection

Poliovirus type 2, PFU/ml

$10^5$  

$10^6$  

$10^7$  

$10^8$
Identification of RNA polymerases

- Polymerase discovered in (-) strand virus particles
- Sequence alignments (GDD), synthesis of recombinant proteins
- Crystal structures
RNA and RdRp in the virus particle

- (-) strand RNA genomes: RdRp, RNA coated with protein (nucleocapsid)
- (+) strand RNA genomes: no RdRp, naked (exceptions: retrovirus, coronavirus)
- dsRNA genomes: RdRp, naked RNA
Nucleocapsids
RNA structure

A

Hairpin loop

Bulge loop

Interior loop

Multibranched loop

Stem

B

L1

S1

5'

L2

S1

L1

S2

3'

C

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Rules for viral RNA synthesis

- RNA genome must be copied end to end with no loss of nucleotide sequence
- Viral mRNAs must be produced that can be efficiently translated by cellular protein synthesis machinery
Universal rules for RNA-directed RNA synthesis

- RNA synthesis initiates and terminates at specific sites on the template
- RdRp may initiate synthesis de novo (like cellular DdRp) or require a primer
- Other viral and cell proteins may be required
- RNA is synthesized by template-directed stepwise incorporation of NTPs, elongated in 5’-3’ direction
- Some non-templated synthesis
Two modes of initiation of RNA synthesis

**De novo initiation**

3'-terminal initiation

3'- N1 N2 5'

NTP  NTP

OH

**Primer-dependent initiation**

Protein primer

Terminal protein

3'- NTP

OH

Capped primer

5' Cap  NTP

OH
Two-metal mechanism of polymerase catalysis
Which is a universal rule about RNA directed RNA synthesis?

A. RdRp may initiate *de novo* or require a primer
B. RNA synthesis initiates randomly on the RNA template
C. RNA is synthesized in a 3’-5’ direction
D. RNA synthesis is always template-directed
Sequence relationships among polymerases

- Gly-Asp-Asp in (+) strand RNA polymerases
- Asp-Asp in RT, segmented (-) strand polymerases
- Gly-Asp-Asn in nonsegmented (-) strand polymerases
Structure of UTP bound to poliovirus RdRp
(+) strand RNA viruses

(+) strand RNA viruses
Flavi- and picornaviruses

(+ strand) genome RNA (mRNA)
Replication

(-) strand full-length complement

(+ strand) genome RNA (mRNA)

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)

(+ strand) genome RNA (mRNA)
Replication

(-) strand full-length complement

mRNA synthesis

(+ strand) genome RNA (mRNA)
**Poliovirus**

* viral genome = mRNA
Viral (+) strand genome

Translation, processing

Capsid

Proteases and RNA synthesis

VPg

5'

UTR

AₙA₀H₃'

UTR

P1

P2

P3

VP0

VP3

VP1

2A

2B

2C

P3

3AB

3CDₚₐₒ

3A

3B

3Cₚₐₒ

3Dₚₒ
Cellular polyadenylated RNAs not copied
Go to:

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

Which is a part of the poliovirus replication strategy?

A. The production of subgenomic mRNAs
B. De novo (without primer) initiation of RNA synthesis
C. Circularization of template for initiation of RNA synthesis
D. All of the above
(+ ) strand RNA viruses

(+ ) strand RNA viruses
Flavi- and picornaviruses

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)

5' C
Replication
3' 5' (+ ) strand genome RNA (mRNA)
5' 3' (- ) strand full-length complement
5' C
(+ ) strand genome RNA (mRNA)

5' C
Replication
3' 5' (+ ) strand genome RNA (mRNA)
5' 3' (- ) strand full-length complement
5' C
mRNA synthesis
5' C
(+ ) strand genome RNA (mRNA)
Togaviridae
viral genome = mRNA
But not all of it is translated!
(-) Strand RNA viruses

Unimolecular

3' \rightarrow 5' \text{ (-) strand genome RNA}

Replication

3' \rightarrow 5' \text{ (+) strand full-length complement}

5' \rightarrow 3' \text{ (-) strand genome RNA}

mRNA synthesis

Segmented

3' \rightarrow 5' \text{ (-) strand genome RNA}

Replication

3' \rightarrow 5' \text{ (+) strand full-length complement}

5' \rightarrow 3' \text{ (-) strand genome RNA}

mRNA synthesis
VSV

*Viral genome is not mRNA*

When the viral genome is NOT mRNA, there must be a switch from mRNA to genome RNA synthesis.
Unimolecular

(-) strand RNA

mRNA synthesis

(+)-strand mRNA

Translation

N
P/C
M
G
L
RNA polymerase binds at 3' end of N gene

Initiation of mRNA synthesis at 3' end of N gene

Synthesize N mRNA and terminate at intergenic region (ig)

Reinitiate at 3' end of P gene
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Influenza virus viral genome is not mRNA

When the viral genome is NOT mRNA, there must be a switch from mRNA to genome RNA synthesis.
Initiation

\[ m^{7}Gpppm^{6}AmpC(m)pAp \ldots \ldots UpUpGpApCp \]

Elongation


Cleavage

\[ \text{(-) strand RNA} \]

\[ UpCpCpUpUpUpUpCp \ldots \]
How are influenza virus and VSV RNA synthesis similar?

A. The switch from mRNA to genome RNA synthesis is controlled by an RNA binding protein
B. Polyadenylation occurs at a short stretch of U residues
C. Viral mRNAs are shorter than (-) genome RNA
D. All of the above
dsRNA viruses

*Reoviridae*: reovirus, rotavirus

Double-stranded RNA viruses

- 5' 3'
- 3' 5'
- (-) strand
- (+) strand
- mRNA synthesis

- 3' 5'
- (+) strand full-length complement (mRNA)
- Translation
- Protein

- 3' 5'
- (+) strand 3'
- (-) strand 5'
- Replication
- Genome RNA
Reovirus
(+) strand not accessible by ribosomes!

The viral genome is not mRNA
Where is the switch to genome synthesis?
Each dsRNA segment is attached to RdRp via the 5′-cap
RNA directed RNA synthesis

• (+) RNA, (-) RNA, dsRNA
• Polymerase basics
• Site of RNA synthesis
• Genome replication
• mRNA synthesis
• poly(A) addition
Next time: Transcription and RNA processing