RNA directed RNA synthesis

Lecture 6
Biology 4310
Virology
Spring 2020

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 - Frankel-Conrat experiment, TMV RNA is genetic material
- By 1959, RNA was identified in many animal viruses
- 1960s - studies on viral RNA replication begin
Identification of RNA polymerases

RNA polymerase activity in infected cells

ATP, UTP, GTP, CTP

Incubate
Measure RNA synthesis

Cell extract

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

B

L1
S1
L2
S2
5' 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.
- There is some non-templated synthesis.
Two modes of initiation of RNA synthesis

**De novo initiation**

3'-terminal initiation

3'– N1 N2 5'

NTP

OH

**Primer-dependent initiation**

Protein primer

3'- Terminal protein

OH

NTP

Capped primer

3'– Cap NTP 5'

OH
Two-metal mechanism of polymerase catalysis
Go to:

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

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
Poliovirus RdRp

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Structure of UTP bound to poliovirus RdRp
(+)-strand RNA viruses

Flavi- and picornaviruses

(+)-strand RNA viruses

\[ \text{Replication} \]

\[ \text{(+)-strand genome RNA (mRNA)} \]

\[ \text{(-)-strand full-length complement} \]

\[ \text{(+)-strand genome RNA (mRNA)} \]

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)

\[ \text{Replication} \]

\[ \text{(+)-strand genome RNA (mRNA)} \]

\[ \text{(-)-strand full-length complement} \]

\[ \text{mRNA synthesis} \]

\[ \text{(+)-strand genome RNA (mRNA)} \]
Poliovirus
viral genome = mRNA
Cellular polyadenylated RNAs not copied
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

Flavi- and picornaviruses

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)

5' C → Replication → (+) strand genome RNA (mRNA)

3' → (-) strand full-length complement → 5'

5' C → Replication → (+) strand genome RNA (mRNA)

3' → mRNA synthesis → 5' C

(+)-strand genome RNA (mRNA)
Togaviridae
 viral genome = mRNA
 But not all of it is translated!
(-) Strand RNA viruses

(-) strand RNA viruses
Unimolecular

5' C

mRNA synthesis

3' ← 5' (-) strand genome RNA

5' ← 3' (+) strand full-length complement

3' ← 5' (-) strand genome RNA

Segmented

5' A → 3'

mRNA synthesis

3' ← 5' (-) strand genome RNA

5' ← 3' (+) strand full-length complement

3' ← 5' (-) strand genome RNA
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

Leader RNA

(+)-strand mRNA

mRNA synthesis

Translation
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
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.
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
full-length complement (mRNA)

mRNA synthesis

Translation

Protein

Replication

Genome RNA

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Reovirus
(+) strand not accessible by ribosomes!

The viral genome is not mRNA
Where is the switch to genome synthesis?
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Each dsRNA segment is attached to RdRp via the 5’-cap.
Next time: Transcription and RNA processing