RNA directed RNA synthesis

Lecture 6
Biology 3310/4310
Virology
Spring 2018

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
Identification of RNA polymerases
Identification of RNA polymerases

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

- (-) strand RNA genomes: coated with protein

- (+) strand RNA genomes: naked (exceptions: retrovirus, coronavirus)

- dsRNA genomes
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
**De novo initiation**

3′-terminal initiation

3′-N1 N2

NTP

NTP

OH

5′

Internal initiation

5′-pppG

3′-AUC AUC AUC UG

5′

Elongation

5′-pppG UAG AC

3′-AUC AUC AUC UG

5′

Slip back

5′-pppG UAG AC

3′-AUC AUC AUC UG

5′

**Primer-dependent initiation**

Protein primer

3′-

NTP

Terminal protein

OH

5′

Capped primer

5′-Cap

3′-

NTP

OH

5′
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

5' C → Replication → (+) strand genome RNA (mRNA) → 5'
3' → 5'

(-) strand full-length complement

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

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)

5' C → Replication → (+) strand genome RNA (mRNA) → 5'
3' → 5'
mRNA synthesis

(-) strand full-length complement

5' C → (+) strand genome RNA (mRNA) → 5'
Poliovirus
viral genome = mRNA
Cleavage

VPg

Poliovirus genome RNA

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cis-acting RNA element (moveable)

Cellular polyadenylated RNAs not copied
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Vesicle formation in virus-infected cells

Uninfected HeLa cell

Flavivirus infected cell

Coronavirus-infected cell

PV-infected HeLa cell
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

Flavi- and picornaviruses

(+) strand RNA viruses

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)
Togaviridae
viral genome = mRNA
But not all of it is translated!
RNA polymerase and accessory proteins

Proteolytic processing

P1234 nsP1 nsP2 nsP3 nsP4

P123 nsP1 nsP2 nsP3

(+) strand RNA

Translation

UTR

(+)

UTR

UTR

(-) strand RNA

3'

UTR

UTR

UTR

Subgenomic mRNA synthesis

(+)

UTR

UTR

Translation/processing

Capsid PE2 6K E1

PE2 6K E1

E3 E2
(-) Strand RNA viruses

Unimolecular

5' - C

3' mRNA synthesis

5' (-) strand genome RNA

Replication

5' (+) strand full-length complement

3' (-) strand genome RNA

Segmented

5' - C

3' mRNA synthesis

5' (-) strand genome RNA

Replication

5' (+) strand full-length complement

3' (-) strand genome RNA
VSV

viral genome is not mRNA
Unimolecular

(-) strand RNA

3' \rightarrow 5'

Leader RNA

5' \rightarrow 3'

(+) strand mRNA

5' \rightarrow 3'

mRNA synthesis

Translation

N

P/C

M

G

L

Glycoprotein (G)
Lipid bilayer
Matrix protein (M)
(-) strand RNA genome coated with Nucleocapsid protein (N)
RNA polymerase (L and P proteins)
**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
mRNA synthesis

(-) strand genome RNA segment

Replication

20 nucleotides

Host m^7Gp primer

5' pppA unprimed

NP

Replication

3'

Appp 5'

Appp 5' unprimed

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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**

- **Genome RNA**
  - 5' (--) strand
  - 3' (+) strand

- **mRNA synthesis**
  - 3' (--) strand full-length complement (mRNA)

- **Translation**
  - Protein

- **Replication**
  - 3' (+) strand
  - 5' (--) strand

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Reovirus
(+) strand not accessible by ribosomes!
Virion

Infectious sub-viral particle (ISVP)

Core

L1  L2  L3

M1  M2  M3

S1  S2  S3  S4

dsRNA

mRNA synthesis

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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