RNA directed RNA synthesis

Lecture 6
Biology 4310
Virology
Spring 2021

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

![Graph showing RNA polymerase activity over time post-infection with different viral types.]
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
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

Primer-dependent initiation

Protein primer

Terminal protein

Capped primer

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

A. RdRp may initiate \textit{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
Poliovirus RdRp
Poliovirus RdRp

Template entry

dsRNA exit

NTP entry

Top

Front

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

Flavi- and picornaviruses

5' C  
3'  
5' C

Replication

(+)-strand full-length complement

(+)-strand genome RNA (mRNA)

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)

5' C  
3'  
5' C

Replication

mRNA synthesis

(-)-strand full-length complement

(+)-strand genome RNA (mRNA)
Poliovirus
viral genome = mRNA
Cellular polyadenylated RNAs not copied
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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

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

Alphaviruses (Togaviridae - Sindbis, SFV, Chik)

5' - Replication - 5' (5')
3' - (+) strand genome RNA (mRNA) - 5'
5' - mRNA synthesis - 5'
5' - (+) strand genome RNA (mRNA) - 5'
**Togaviridae**

viral genome = mRNA

But not all of it is translated!
Coronaviruses

1. Entry
2. Uncoating
3. Replication
4. mRNA Synthesis
5. Viral Proteins Synthesis
6. Assembly
7. Assembly
8. Maturation
9. Maturation
10. Maturation
11. Release

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CoV RNA synthesis

This mechanism of mRNA synthesis allows for high rates of recombination among CoVs.
(-) Strand RNA viruses

Unimolecular

5' C

mRNA synthesis

3' < 5' (-) strand genome RNA

Replication

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

3' 5' (-) strand genome RNA

Segmented

C

mRNA synthesis

3' < 5' (-) strand genome RNA

Replication

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.
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
Segmented (-) strand RNA coated with nucleocapsid protein (NP)

(-) strand RNA genome segments

mRNAs

Translation

Splicing

Translation
Activation of influenza virus RNA polymerase

Inactive polymerase

Cap-binding

Cap-binding and 3' (-) strand genome RNA binding sites activated

Incorrect 3' sequence

Proper 3' sequence on (-) strand genome

Endonuclease inactive

Endonuclease activated; initiation and elongation occur
**Initiation**

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

**Elongation**


**Cleavage**

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

\[ \text{UpCpCpUpUpUpUpCp...} \]

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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' full-length complement (mRNA)

- Translation
  - Protein

- Replication
  - (+) strand
  - (-) strand

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Reovirus

(+) strand not accessible by ribosomes!

The viral genome is not mRNA
Where is the switch to genome synthesis?
Release of mRNA from rotavirus particles

Each dsRNA segment is attached to RdRp via the 5’-cap
Origins of diversity among RNA viruses

- Misincorporation of nucleotides
  - Lack of proofreading activity in RNA dependent RNA polymerase: high error frequencies (1 misincorporation / $10^3 - 10^4$ nt polymerized)
  - Average error frequency: 1 in $10^4$ or $10^5$ nucleotides polymerized
  - In a 10 kb RNA virus genome, a mutation frequency of 1 in $10^4$ results in about 1 mutation per genome
**Nidovirales are an exception**

- exoN protein is a 3’-5’ exonuclease that corrects RNA polymerase errors
- Removal of exoN produces a virus with 15-20 fold increase in mutation rate
- ExoN may allow faithful replication of large (up to 41 kb) RNA genomes
Major fidelity checkpoint for poliovirus RdRp

- Determined by how template, primer, NTP interact at active site
- NTP first binds in a way that does not allow ribose to interact with Asp-Asn amino acids
- If NTP is correctly base paired, conformational change in enzyme occurs which reorients triphosphate, allows phosphoryltransfer to occur
Fidelity control by poliovirus RdRp

- $3D_{pol}$ single amino acid change, G64S, makes fewer errors
- Slows conformational change that occurs on NTP base pairing, reducing elongation rate
- AA 64 is in fingers domain, remote from active site but change makes enzyme more dependent on correct NTP base pairing in active site, increasing fidelity
- Mechanism likely conserved among RdRps
RNA recombination

- Exchange of nucleotide sequences among different genomic RNA molecules (distinct from reassortment)
- Shapes RNA virus world by rearranging genomes and creating new one
- Can be relatively high: 10-20% of poliovirus RNA molecules recombine in a single replication cycle
Recombination control by poliovirus RdRp

- $3D^{\text{pol}}$ single amino acid change, L420A, reduced recombination frequency
- Located in thumb domain, RNA exit channel
- Reduces initiation rate and stability of elongation complexes, no affect on fidelity
Next time: Transcription and RNA processing