RNA Synthesis

Lecture 6
Biology W3310/4310
Virology
Spring 2014

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

Assays: cell extracts incubated with NTPs
Identification of RNA polymerases

- Assays: cell extracts incubated with NTPs
- ActD resistant cytoplasmic RNA synthesis in mengovirus/poliovirus infected cells
- Virion polymerase discovered in (-) strand viruses
- Identification by sequence alignments (GDD), synthesis of recombinant proteins
- Crystal structures of many RNA polymerases have been determined
Nature of the RNA template

• (-) strand RNA genomes: coated with protein; ready to begin RNA synthesis upon entry

• (+) strand RNA genomes: naked, ready to be translated upon entry (exception: retroviral, coronaviral)

• dsRNA genomes: cannot be copied into mRNA by the cell; virions contain RNA polymerase
VSV N protein bound to RNA
• RNA genome must be copied end to end with no loss of nucleotide sequence

• Production of viral mRNAs that can be efficiently translated by cellular protein synthetic 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
- Non-templated RNA synthesis
**De novo initiation**

3'-terminal initiation

Capped NTP

Elongation

Slip back

**Primer-dependent initiation**

Protein primer

Capped primer
Sequence relationships among polymerases

- Gly-Asp-Asp in all (+) strand RNA polymerases
- Asp-Asp in RT, segmented (-) strand polymerases
- Gly-Asp-Asn in nonsegmented (-) strand polymerases; birnaviruses have Ala-Asp-Asn
Structure of UTP bound to poliovirus RdRp
cellular polyadenylated RNAs not copied
Cleavage

VPg

Poliovirus genome RNA
Transfer to 3'-end of poly(A)

Priming

Elongation

Synthesis of (-) strand
Vesicle formation in poliovirus-infected cells

Uninfected HeLa cell

PV-infected HeLa cell
(+) strand RNA viruses
Flavi- and picornaviruses

(Togaviridae - Sindbis, SFV, Chik)
(-) strand RNA viruses

Unimolecular

5' [-] 3' mRNA synthesis

3' - 5' Replication

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

3' - 5' (-) strand genome RNA

Segmented

5' [-] 3' mRNA synthesis

3' - 5' Replication

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

3' - 5' (-) strand genome RNA
Unimolecular (-) RNA

(-) strand RNA

3' \rightarrow \text{mRNA synthesis} \rightarrow 5'

(l) RNA

5' \rightarrow 3'

(+)-strand mRNA

AA(G)\text{3'}

A(A)\text{3'}

A(A)\text{3'}

A(A)\text{3'}

A(A)\text{3'}

Translation

N

P

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
Initiation

m7Gpppm6AmpC(m)pAp......UpUpGpApCp...

Cleavage

13

(−) strand RNA

UpCpCpUpUpUpGp...

Elongation

Double-stranded RNA viruses

Reoviridae: reovirus, rotavirus
Each dsRNA segment is attached to pol via the 5’-cap
RNA synthesis as a source of viral diversity

- Mutation
- Recombination
- RNA editing
Mutation

- Lack of proofreading activity in RNA dependent RNA polymerase: high error frequencies (1 minincorporation/10^3 - 10^4 nt polymerized)
- Single amino acid change in poliovirus RdRp causes increased fidelity
- This mutation reduces viral fitness and virulence under selective pressure
Recombination
RNA editing

Non-templated: measles, mumps virus
RNA editing produces mRNA for Ebola virus glycoprotein