Replication of RNA Viruses

Replication: molecular events between uncoating and virus assembly

Replication: general term includes transcription, translation, replication of genomes
RNA Viruses

Positive-Stranded RNA Viruses
- Picornaviruses*
- Togaviruses
- Flaviviruses
- Coronaviruses

Negative-Stranded RNA Viruses
- Nonsegmented
  - Rhabdoviruses
  - Paramyxoviruses
  - Filoviruses
  - Bornaviruses
- Segmented
  - Myxoviruses (influenza)*
  - Bunyaviruses

Ambisense RNA Viruses – Arenaviruses

Ds RNA Viruses -- reovirus (segmented)

Retroviruses --- covered in lectures by P Clapham
Model System for positive-stranded RNA replication

Picornaviruses
Genome of Picornaviruses

7.5 x 10^3 bases

What is the coding capacity of the genome?
How many codons/aa?

How many proteins can the genome encode?
What is average size of a protein?
How is genomic RNA expressed?

Steady state labeling of infected cells
   Sum of molecular weights of polypeptides
detected exceeded coding capacity by 3x

Pulse-chase analysis of proteins in infected cells

results
conclusion
Gene Order and Translation of Picornavirus Virion RNA
How determine order of proteins?

RNA genome

Translation

Polyprotein

VP4 VP2 VP3 VP1
2A 2B 2C
3A 3B 3C 3D
What is responsible for proteolytic cleavages?

1. Translation in reticulocyte extracts
2. Translation in E. coli extracts
3. Antibodies

Conclusions??
Why is genome expressed as a polyprotein?

Properties of most eucaryotic mRNAs

- number of ORFs
- 5' end
- 3' end
- position of AUG

Hypothetical Picornavirus genome

Model mRNA

How would these mRNAs be Translated?
Eucaryotic Initiation of Protein Synthesis

1. eIF4F (3 subunits) binds to cap

2. eIF3+40S complex binds 4G

3. eIF4F complex binds the cap on mRNA

4. Complex (eIF4F-eIF3-ribosome 40S) scans mRNA to first AUG

5. 60S joins complex at the AUG and translation begins
Evidence

1. Caps necessary for translation

2. mRNA ends are necessary for initiation of protein synthesis

3. Hairpin structures (between 5’ end and AUG) inhibit initiation.

4. Internal initiation very inefficient in most mRNAs i.e. first AUG is used to begin protein synthesis and this AUG is usually 20-50 bases from 5’ end

Conclusion--single picornavirus mRNA requires one ORF cleavages generate different proteins
Unique Features of Picornavirus mRNA

1. 5' end no Cap
   Virion RNA   VpG-pUpUpAp------
   mRNA       pUpUpAp------

2. Initiation AUG is not the first
   Rhino   650 bases from 5’ end
   Polio   750 bases from 5’ end (9th AUG)

3. 5' UTR has many hairpins (before AUG used)
Experiments to understand unique properties of virion RNA as a mRNA

1. Deletion analysis of 5' UTR
   135-556 sequence required for initiation at nucleotide 750

2. Effect of 5'UTR sequences on internal translation in an artificial mRNA
Effect of 5'UTR sequences on internal translation in an artificial mRNA

Sequences defined as IRES
Properties of IRES

1. Primary sequences—nothing in common

2. Secondary structures—similar structures
   Different groups-----polio and rhino--type 1
   cardio and aphtho--type 2
   hepato--type 3

3. Host Factors required for function
Fields Virology
Summary of Cap dependent and independent Initiation of protein synthesis
Picornaviruses infection shuts off host protein synthesis.
Translation of capped mRNAs in uninfected and infected cells

Uninfected cells

Infected cells

No translation
Translation in Uninfected and Infected cells

**Conclusion:** infection inhibits cap dependent translation
Mechanism of shut down of capped mRNA translation--
defects in EIF4F

Fields Virology
Replication-Synthesis of RNA

Single Stranded RNA genome
+ strand

Synthesis - strands

Synthesis + strands

Synthesis - strands
Replication

Requires
- 3D=RNA dependent RNA polymerase
- VpG
- host factor (HF)
- UTP

1. \[ 3D-VpG-HF+UTP \rightarrow UUUVpG \]

2. \[ UUAA \rightarrow AAAAn (+) \]

3. \[ AAUU \rightarrow VpGUU (--) \]
Transition from translation to replication
Synthesis of + strands (progeny genomes) and amplification of progeny genomes

Synthesis of + strands

Translation

Proteolytic cleavage

Synthesis of - strands

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Synthesis of + strands
Summary of Picornavirus Replication Cycle

Replication Totally in cytoplasm
Positive Stranded RNA virus recombination

Implications for Live attenuated vaccines

Copy choice
Variations on Theme:

Togaviruses

Two ORFS

Coronaviruses

Seven to eight ORFS

Mechanisms for expression of other ORFS?
Model System for negative-stranded RNA viruses

*Influenza*

Segmented, negative-stranded RNA genome
Influenza Viruses

Genome Structure---8 segments
Replication--overview

Single Stranded **Segmented** RNA Genome

Negative Polarity

- genome

+ mRNA

Primary transcription

Translation

Secondary transcription

Synthesis + strands

Synthesis - strands

Synthesus + strands
How is first step of infection, primary transcription, accomplished (source of enzyme)?

Demonstration of polymerase activity

\[ \text{Virus+Triton X-100+NTP} \rightarrow \text{RNA synthesis} \]
RNA products of the Virion Reaction compared to mRNA in infected cells

1. Size of product—slightly smaller

2. 5' ends of product—no cap

3. Efficiency of synthesis—very low

4. Cell extracts added to the virion reaction
   a. reaction significantly increased transcription
   b. Properties of transcripts like cellular mRNAs
   c. Identification of extract components that stimulated transcription

How define component of cell extracts responsible???
Mechanism of Cap Snatching

[Diagram of the mechanism of Cap Snatching]

Fields Virology
Cell Sites of Influenza Virus Replication

RNA Synthesis: nucleus

Protein Synthesis: cytoplasm
Influenza RNA Synthesis Occurs in the Nucleus

Transport of Genome Into nucleus

NLS in NP bind Karyopherin $\alpha$

Fields Virology
Transcription of genomic RNA

Note termination
And polyA addition
ORFs in transcripts

PB2: component of RNA polymerase, cap recognition
PB1-F2: pro-apoptotic activity
PB1: component of RNA polymerase, endonuclease activity, elongation
PA: component of RNA polymerase, protease
HA: surface glycoprotein, receptor binding, fusion activity, major antigen
NP: RNA binding, RNA synthesis, RNA nuclear import
NA: surface glycoprotein, neuraminidase activity
M1: matrix protein, interaction with vRNPs and surface glycoproteins, nuclear export, budding
M2: membrane protein, ion channel activity, assembly
NS1: multi-functional protein, viral IFN antagonist
NEP/NS2: nuclear export of vRNPs
Replication: Generation of + RNA intermediates
Progeny Genomes Exported from Nucleus

Newly made M1, NEP/NS2, and NP transported into the nucleus
Assembled with genomic RNAs
RNPs exported
Summary of Influenza Replication Cycle

- Adsorption
- Endocytosis
- Low pH
- Fusion and uncoating
- Posttranslational processing
- Translation
- vRNA (-)
- cRNA (+)
- mRNA
- Packaging
- Budding

Fields
Virology
Protocol for Reverse Genetics of Influenza Viruses

Protein expression plasmids (4)

\[ \text{pol II} \rightarrow \text{cDNA (+)} \]

PB1, PB2, PA, NP

vRNA expression plasmids (8)

\[ \text{pol I} \rightarrow \text{cDNA (-)} \]

PB1, PB2, PA, HA, NP, NA, M, NS

Ribozyme or pol I terminator

Transfectant virus
Variations on theme of negative stranded RNA Viruses:

Non-segmented, negative stranded RNA viruses

- Rhabdoviruses
- Paramyxoviruses
- Filoviruses
- Bornaviruses

Replication exclusively in the Cytoplasm except Bornaviruses
Replication

Single Stranded \textbf{non-segmented} RNA Genome
Negative Polarity

Virion associated polymerase

Primary transcription

Translation

Secondary transcription

Synthesus - strands

Synthesis + strands

Synthesis + strands
Gene order  rhabdoviruses, paramyxoviruses
Transcription of Non-segmented Negative Stranded RNA Viruses
Bornaviruses:

Different from other nonsegmented negative stranded RNA viruses

Replication in nucleus

Splicing of transcripts
How define sense of genomic RNAs?

- Polysomes
- Infectious RNA
Reading

Fields Virology

Chapter 5

Sections of Chapters 24 and 47 that discuss replication