Reverse transcription and integration

Lecture 9
Biology W3310/4310
Virology
Spring 2016

“One can’t believe impossible things,” said Alice.
“I dare say you haven’t had much practice,” said
the Queen. “Why, sometimes I’ve believed as many
as six impossible things before breakfast.”
--LEWIS CARROLL, Alice in Wonderland
Tumor virus history

• 1908 - Discovery of chicken leukemia virus, Bang & Ellerman
• 1911 - Discovery of Rous sarcoma virus, Peyton Rous (Nobel Prize 55 years later)
• Called tumor viruses
• Found to have RNA genomes
Temin’s insight

• Retroviruses caused permanent changes in cells (transformation)
• Retroviral DNA was integrated into host genome
• Became permanent part of host DNA
• Provirus
Baltimore and Temin independently discovered RT in RNA tumor virus particles

Listen to TWiV #100 (Baltimore) for more insight
Reverse transcriptase

- Enzyme that countered *Central Dogma*:
  DNA => RNA => protein

- Retroviruses got their name because of their ability to reverse the flow of genetic information
Viruses with RT

- Poliovirus
- Sindbis virus
- Influenza virus
- VSV
- Reovirus
- Hepatitis B virus
Simple genome (ALV)

Proviral DNA

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<tr>
<th>Core</th>
<th>Enzymes</th>
<th>Envelope</th>
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<td>pol</td>
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<tr>
<td>P10</td>
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Genome expression

Genomic RNA, Gag-Pol mRNA, pre-mRNA

1. **Gag precursor**
   - Gag mRNA
   - Translation
   - Mature, viral PR-processed proteins

2. **Gag-Pol precursor**
   - RT (α, β)
   - Mature, viral PR-processed proteins

3. **Singly spliced Env mRNA**
   - Envelope precursor
Reverse transcriptase

- Primer can be DNA or RNA
- Template can be RNA or DNA
- Only dNTPs, not rNTPs, are incorporated
RT

- Bacteria and Archaea have RT activity
- Therefore RT evolved before the separation of Archaea, bacteria, and eukaryotes
- RT might be the bridge between early RNA world and modern DNA world
- RT also in HBV, *Caulimoviridae*
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
RNAse H: A second activity of RT

- Cleaves RNA only when in duplex form
- RNA can be in RNA:RNA or RNA:DNA duplexes
- Makes endonucleolytic cleavages
- Produces short oligonucleotides with 5’-phosphate, 3’-OH
DNA synthesis is slow (4 h per 9 kb genome) and error prone (1 misincorporation per $10^4$ to $10^6$ nt).
Reverse transcriptase has revolutionized molecular biology. Which statement about the enzyme is not correct?

1. RT is unique to retroviruses
2. RT is packaged in the retrovirus particle
3. The RT protein also has RNAse H activity
4. The name of the enzyme comes from its ability to reverse the flow of genetic information
5. Might have bridged the ancient RNA world and the DNA worlds
Coated with NC protein
50-100 molecules RT per virus particle
RNA dimer

- Explains why retroviruses are relatively resistant to UV and ionizing radiation
- Two copies of all genes
- Copy-choice rebuilds one functional genome
DNA synthesis: cytoplasmic

Initiation of (−) strand DNA synthesis
First template exchange

R-r annealing

ppt u3 r

\(A_r^\text{A}_{\text{OH3'}}\)

ppt = polypurine tract
(+) strand DNA synthesis
Second template exchange is facilitated by annealing of PBS sequences.
Which of the following steps occur during reverse transcription of retroviral genomic RNA?

1. Priming of (-) DNA synthesis by tRNA
2. Two template exchanges
3. Degradation of the viral RNA by RNase H
4. Generation of two LTRs
5. All of the above
Preference for integration into DNA sequences that are wrapped around a nucleosome
• One DNA produced from two RNAs by RT

• Strong promoter (the LTR) built during RT

• Proviral DNA directs the host transcription machinery to synthesize many copies of viral mRNA

• Viral mRNA is translated into viral proteins OR encapsidated into virus particles

There is no DNA replication and no RNA replication
Integration of retroviral DNA has which of the following properties:

1. Catalyzed by a viral enzyme
2. Occurs on all chromosomes but preferentially at sites that are wrapped around a nucleosome
3. Leads to the formation of a provirus
4. Leads to production of viral mRNAs by host pol II
5. All of the above
• No mechanism for precise excision of integrated provirus
• Only way out of genome is transcription by host RNA pol II
• Genomes are littered with ancient and modern retroelements
Retroelements

• Sequences that move in the genome via RT
• Proviral DNA integrated into the germline = endogenous retroviruses, ERV
• Often replication-defective
• ~42% of human genome comprises mobile genetic elements, including endogenous proviruses and other retroelements
Rescue of an endogenous human retrovirus

- HERV-K, infected human ancestors <1 Myr ago
- Repaired mutations

Reconstitution of an infectious human endogenous retrovirus.
Lee YN, Bieniasz PD.
A retrovirus makes chicken eggshells blue

Which of the following statements about retroelements is not correct?

1. There are many copies in eukaryotic genomes
2. They are currently entering the Koala germline
3. Those in the human genome produce infectious viruses
4. They can be beneficial
5. None of the above
Hepadnaviridae

A

Viral particle
Viral DNA
Capsid
Large (L)
Medium (M)
Small (S)

Polymerase (P)

42 nm

Incomplete particles

20 × 20–200 nm

15–25 nm

B

Pregenome RNA
ORF S
ORF C
Pre-C
ORF P
ORF X

Pre-S1
Pre-S2

2.1 kb
2.4 kb
3.4 kb
10.7 kb

(+)
(-)

DR1
DR2

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No genome integration