Viral DNA replication

Lecture 7
Biology W3310/4310
Virology
Spring 2014

The more the merrier
--ANONYMOUS
Viruses must replicate their genomes to make new progeny

- Parvovirus
- Hepatitis B virus
- Adenovirus
- Herpesvirus
- Polyomavirus
- Papillomavirus
- Poxvirus

Diagram:

1. $\pm$ DNA to $+$ DNA
2. $+$ DNA to $+$ mRNA
3. $\pm$ RNA to $\pm$ DNA
4. $+$ mRNA to $+$ mRNA
5. $-$ RNA to $+$ mRNA
6. $+$ RNA to $-$ DNA

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Universal rules of DNA replication

- DNA is synthesized by template-directed incorporation of dNMPs into 3’-OH of DNA chain
- DNA is always synthesized 5’-3’ via semiconservative replication (two daughter strands)
- Replication initiates at specific sites on template called **origins**
- Catalyzed by DdDp + accessory proteins
- Primer-dependent
What’s the host for?

• Viral DNA replication always requires synthesis of at least one viral protein, sometimes many (hence always delayed after infection)

• Simple viruses require more host proteins - genetic economy

• Complex viruses encode many, but not all proteins required for replication
Where does the polymerase come from?

- Small DNA viruses do not encode an entire replication system
  - Encode proteins that orchestrate the host
    - Papillomaviridae, Polyomaviridae, Parvoviridae
- Large DNA viruses encode most of their own replication systems
  - Herpesviridae, Adenoviridae, Poxviridae
Viral proteins

• DNA polymerase and accessory proteins
• Origin binding protein, helicases
• Exonucleases
• Enzymes of nucleic acid metabolism (thymidine kinase, ribonucleotide reductase, dUTPase)
Diverse viral genome structures

Parvoviridae (4–6 kb)

Polyomaviridae (5 kbp)

Herpesviridae (120–220 kbp)

Adenoviridae (36–48 kbp)

Poxviridae (130–375 kbp)
Two mechanisms of dsDNA synthesis

Replication fork
- Papillomaviruses
- Polyomaviruses
- Herpesviruses
- Retroviral proviruses

RNA primers

Strand displacement (primer)
- Adenoviruses (protein)
- Parvoviruses (DNA hairpin)
- Poxviruses (DNA hairpin)

Never RNA primed
The 5’-end problem

DNA template

RNA primers

3’

5’

5’

3’

Now what?
Lessons from SV40

A

B

Polyomaviridae (5 kbp)

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Semi-discontinuous DNA synthesis from a bidirectional origin

No end problem!
Recognition and unwinding of SV40 origin

SV40

LT binding site II

EP

1

ATP + LT

2

Conformational change of EP sequence

3

ATP

ADP + Pi

Rpa

Topo I

LT

LT

T has 3’-5’ helicase activity

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Synthesis of leading and lagging strands

Rf-C binds 3’OH along with PCNA and pol δ
—RF-C a clamp loading protein
—Allows entry of PCNA on DNA
—Causes release of pol α
Form sliding clamps along DNA

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Synthesis of leading and lagging strands
An SV40 replication machine
# Cell proteins required for polyomavirus DNA replication

<table>
<thead>
<tr>
<th>Protein</th>
<th>Synonym(s)</th>
<th>Contacts</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rp-A</td>
<td>Rf-A</td>
<td>Primase</td>
<td>Binds to single-stranded DNA; origin unwinding in cooperation with LT</td>
</tr>
<tr>
<td>ssB</td>
<td></td>
<td>LT</td>
<td></td>
</tr>
<tr>
<td>DNA polymerase α-primase</td>
<td>Polo/primase</td>
<td>LT</td>
<td>Synthesis of RNA primers and Okazaki fragments on leading and lagging strands</td>
</tr>
<tr>
<td>Rf-C</td>
<td>Activator 1</td>
<td>Pcna</td>
<td>ATP-dependent clamp loading; also required for release of Pcna from DNA</td>
</tr>
<tr>
<td>Pcna</td>
<td></td>
<td>Rf-C</td>
<td>Sliding clamp</td>
</tr>
<tr>
<td>DNA polymerase δ</td>
<td>Pcna</td>
<td></td>
<td>Processive synthesis of leading and lagging strands when bound to Pcna</td>
</tr>
<tr>
<td>Rnase H1</td>
<td></td>
<td></td>
<td>Endonucleolytic degradation of RNA base-paired with DNA; removal of RNA primers</td>
</tr>
<tr>
<td>Fen1</td>
<td></td>
<td></td>
<td>5′→3′ exonuclease; removal of RNA primers</td>
</tr>
<tr>
<td>DNA ligase I</td>
<td></td>
<td></td>
<td>Scaling of daughter DNA fragments</td>
</tr>
</tbody>
</table>
Function of topoisomerases

A

Covalently closed circular template

DNA replication → Unwound parental duplex

Over wound region

Topoisomerase I or II → One strand cleaved

Relaxed supercoils

B

DNA replication → Over wound region

Topoisomerase II → Both strands cleaved

ATP → ADP + P_i


DNA priming: Parvoviruses
• Replication is continuous
• No pol α, uses ITR to self-prime
• Requires pol δ, RF-C and PCNA
• Rep78/68 proteins are required for initiation and resolution: endonuclease, helicase, binds 5’-terminus
• No replication fork, strand displacement
Protein priming: Adenovirus

- Origins at both ends
- Strand displacement synthesis
- Semiconservative DNA replication
Protein priming: Adenovirus

Ad DNA pol links α-phosphoryl of dCMP to OH of Ser residue only when pTP is assembled with DNA pol into preinitiation complex at ori.
Adenoviral ssDNA binding protein
Viral origins

- AT-rich segments recognized by viral origin recognition proteins
- Seed assembly of multi-protein complexes
- Some viral genomes have one ori; others up to 3
Viral origins of DNA replication

SV40

Enhancer  Spl-binding sites

Core origin

AT-rich element

LT-binding site II

Early imperfect palindrome

LT-binding site I

HSV-1 OriL

UL29

UL30

Ad2

Pol- pTP

Nf-1

Oct-1

Core origin

Domain B

Domain C

10 bp

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Viral origin recognition proteins

- Polyomavirus T binds specifically to DNA
- Papillomavirus E1 binds ori in presence of E2
- Parvovirus Rep68/78 binds at ends and unwinds DNA, also involved in terminal resolution
- Adenovirus pTP binds at terminus and recruits DNA pol
- Herpesvirus UL9 protein recruits viral proteins to AT-rich ori and then unwinds DNA
SV40 large T

- T is a species-specific DBP/OBP
  - Pre-initiation complexes do not form in the wrong species
  - Failure to interact with DNA pol α - primase

- Binds and sequesters cell cycle regulators
  - Causes cells to enter S phase
Big DNA viruses: Herpes simplex virus

- 2 oriS and a unique oriL sequence
- DNA enters as a linear molecule and converts to circle
- Replicates as rolling circle
Initiation of herpesvirus DNA replication

Host proteins are responsible for circularization
Rolling circle replication
HSV gene products required for replication

- UL5, 8 and 53 - form primase
- UL42 - a processivity protein
- UL9 - origin binding protein
- UL29 - ssDNA binding protein
- UL30 - DNA polymerase
Poxvirus

- All viruses discussed replicate in nucleus
- Poxviruses replicate in cytoplasm
- DNA synthesis is independent of cell proteins
Poxvirus DNA factories

DNA

DNA binding protein

merge
Poxvirus DNA replication

1. Nick, unwind
2. Extend 3’ end
3. Refold hairpin ends
4. Extend 3’ end
5. Extend 3’ end
6. Concatamer resolution

# Poxvirus DNA replication enzymes

<table>
<thead>
<tr>
<th>Function</th>
<th>Protein</th>
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</table>
| DNA replication, repair, recombination | DNA polymerase  
DNA pol processivity protein  
DNA primase  
Topoisomerase I  
ssDNA binding protein  
DNA ligase  
Holliday junction resolvase  
Protein kinase (BAF antagonist)  
Multifunctional ‘scaffold’ protein  
Uracil DNA glycosylase  
dUTPase  
dsDNA break repair |
| Nucleotide metabolism            | Thymidine kinase  
Thymidylate kinase  
Ribonucleotide reductase        |
Regulation of DNA synthesis

• Most of our cells do not divide or do so rarely
• Viruses do not replicate well in quiescent cells
• Viruses must induce host replication proteins
• Done by virus encoded immediate early and early gene products
- Cellular retinoblastoma (rb) gene
- Rb protein controls entry into S
- Rb loss associated with tumors = tumor suppressor gene
Abrogation of Rb by viral proteins

SV40 LT
HPV-16 or -18 E7
Ad2 E1A

Increased synthesis of cellular and some viral replication proteins

Inhibition of synthesis of cellular and some viral replication proteins

Cell cycle-specific kinases

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Regulating viral DNA synthesis

- In latently infected cells (no or few virions produced), DNA synthesis is low.
- When virions are made, DNA synthesis is exponential.
- EBV: different origins are used for low (OriP) or exponential replication (OriL).
- HPV: One origin for both states.
Replicating once per cell cycle: EBV OriP
HPV: One ori for controlled and exponential replication