Viral DNA replication

Lecture 8
Biology W3310/4310
Virology
Spring 2015
Viruses must replicate their genomes to make new progeny
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?
Viruses can’t do it themselves

• 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)
Which statement about viral DNA synthesis is NOT correct?

1. Large DNA viruses encode many proteins involved in DNA synthesis
2. Small DNA viruses encode at least one protein involved in DNA synthesis
3. Viral DNA replication is always delayed after infection because it requires the synthesis of at least one viral protein
4. Some viruses encode all proteins needed for DNA replication
Diverse viral genome structures

A  Adenovirus-associated virus type 2 (parvovirus), 4680 bp

B  Simian virus 40 (polyomavirus), 5234 bp

C  Human adenovirus Type 5, 35,937 bp

D  Herpes simplex virus type 1 (Herpesvirus), ~150 kbp

E  Vaccinia virus (poxvirus), ~200 kbp

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Two mechanisms of dsDNA synthesis

- Replication fork
  - Papillomaviruses
  - Polyomaviruses
  - Herpesviruses
  - Retroviral proviruses

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

- RNA primers
- Never RNA primed
The 5’-end problem

RNA primers

DNA template

 elongate

 excise primers, elongate, ligate

Now what?
Lessons from SV40

A

B

Polyomaviridae (5 kbp)

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Semi-discontinuous DNA synthesis from a bidirectional origin

A

Replication fork
5' 3'
3' 5'
Ori

Leading 3'
Lagging 3'

Leading 3'
Lagging 3'

Replication fork
3' 5'

B

5' 3'
3' 5'
RNA

5' 3'
3' 5'
RNA +

5' 3'
3' 5'
Gap

No end problem!
Recognition and unwinding of SV40 origin

SV40

LT binding site II

EP

Fig. 18.2: Recognition and unwinding of SV40 origin (LT has 3’-5’ helicase activity)
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

Synthesis of RNA primers
Synthesis of short DNA fragments

Synthesis of long DNA
Synthesis of leading and lagging strands
An SV40 replication machine
Function of topoisomerase

Covalently closed circular template → DNA replication → Unwounded parental duplex → Overwound region → During replication: Topoisomerase I or II → One strand cleaved → Relaxed supercoils

DNA replication

After replication: Topoisomerase II → Both strands cleaved

ATP → ADP + Pi

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The SV40 genome is a circular dsDNA. Which statement about its replication is correct?

1. Viral T antigen binds and unwinds the ori
2. Replication is bidirectional from a single ori
3. The 5’-end problem is solved
4. Has leading and lagging strand synthesis
5. All of the above
DNA priming: Parvoviruses

The diagram shows the replication region (rep ORF) and capsid ORF (cap ORF) of a parvovirus genome. The replication origin (ori) is indicated, as well as the genes p5, p19, and p40. The DNA sequence is also shown, with the primer binding site (TR) and the primer annealing site (ori) highlighted.
• 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

No end problem!
Adenoviral ssDNA binding protein
Go to:

m.socrative.com
room number: virus

How is DNA replication of parvovirus and adenovirus similar?

1. They both require protein-linked primers
2. Replication occurs by strand displacement
3. DNA synthesis occurs in the cytoplasm
4. A replication fork occurs in both
5. None of the above
Herpes simplex virus

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

- 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

1. Nick
2. Continuous DNA synthesis
3. Discontinuous DNA synthesis
4. Concatemer

Genome length

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- 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
At least 15 viral proteins involved in viral DNA synthesis
What makes poxvirus DNA replication different from all of the other viruses we discussed today?

1. The complete replication machinery is encoded by the viral genome
2. DNA synthesis occurs in the nucleus
3. DNA synthesis occurs by strand displacement
4. None of the above
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

HSV-1 OriL

Ad5

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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
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
Ad5 E1A

Increased synthesis of cellular and some viral replication proteins
(needed for DNA synthesis, and to pass through cell cycle)

Inhibition of synthesis of cellular and some viral replication proteins
HPV: One ori for controlled and exponential replication

- Keratinized dead skin cells
- Epidermal cells
- Basal lamina

More differentiated skin cells

Precursor skin cells

More differentiated skin cells

Productive replication in differentiated cells

Maintenance Replication

Limited amplification of episomal papillomaviral DNA

Papillomavirus Infection

$x \times 10^3$