DNA Replication

• Viruses must replicate their genomes to make new progeny

• This always requires expression of at least one virus protein, sometimes many (hence always delayed after infection)

• DNA is always synthesized 5’ – 3’

• Replication initiates at a defined origin (Ori) using a primer

• The host provides other proteins
The 5' end problem

RNA primers

DNA template

3'  5'

5'  3'

3'  5'

3'  5'

5'  3'

excise primers, elongate, ligate

Now what?
Two Basic Modes of Replication

- Papillomaviruses
- Polyomaviruses
- Herpesviruses
- Retroviral proviruses

- Adenoviruses (protein)
- Parvoviruses (DNA hairpin)
- Poxviruses (DNA hairpin)
Where Does the DNA Polymerase Come From?

• Small DNA viruses do not encode an entire genome replication system
  -encode proteins that orchestrate the host
  -Papillomaviridae, Polyomaviridae, Parvoviridae

• Large DNA viruses encode most of their own replication systems
  -Herpesviridae, Adenoviridae, Poxviridae
A dsDNA genome: Polyomaviridae, Adenoviridae, Herpesviridae, Poxviridae

B Polyomaviridae (5 kbp)

C Adenoviridae (36–48 kbp)

D Herpesviridae (120–220 kbp)

E Poxviridae (130–375 kbp)
Protein Priming

1. Protease
2. DBP, dNTPs
3. Single-stranded DNA Template
4. ITRs
5. Displaced ss Template DNA
6. pTP-Pol
Lessons from bacteriophage $\phi 29$
Adenoviral DBP
Replication of adenovirus genome

- An example of strand displacement synthesis
- Utilizes a protein primer
- Origin is at both ends
- DNA polymerase is viral
- Other viral proteins involved: terminal protein, ssDNA binding protein
- Viral early proteins (E1a) induce quiescent cells into S phase
Adenovirus DNA replication requires cellular proteins
A dsDNA genome: Polyomaviridae, Adenoviridae, Herpesviridae, Poxviridae

B Polyomaviridae (5 kbp)

C Adenoviridae (36–48 kbp)

D Herpesviridae (120–220 kbp)

E Poxviridae (130–375 kbp)
Herpes simplex virus

- HSV has 2 identical oriS and a unique oriL that is active in terminally differentiated neurons – role in transition from latent to productive infection?
- DNA enters as linear molecule converts to circle
- Replicates as a rolling circle
Viral proteins for Herpes simplex virus genome replication

- UL5, 8 and 52 - form primase, helicase
- UL42 - a processivity protein
- UL9 - Origin Binding Protein
- UL29 - ssDNA Binding Protein
- UL30 - DNA polymerase
- 5 enzymes of nucleic acid metabolism, such as TK
- Necessary but not sufficient!
Poxviruses

- All viruses discussed replicate in nucleus
- Poxvirus - cytoplasmic factories - DNA synthesis is independent of cellular proteins

*Poxviridae* (130–375 kbp)
Vaccinia DNA factories

DNA only  I3 only  merge

DNA stained blue; viral DNA binding protein (I3) stained red
Vaccinia DNA factories

DNA stained blue; viral DNA polymerase (E9) stained red
Poxvirus DNA replication

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

FIGURE 15.5 A model for vaccinia virus DNA replication. See text for details. Newly synthesized DNA is shown in orange.

### Poxvirus DNA replication enzymes

<table>
<thead>
<tr>
<th>Function</th>
<th>Protein</th>
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| DNA replication, repair, recombination | DNA polymerase*  
DNA pol. processivity factor  
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

* Genes coding for these proteins have been shown to be required for carrying out viral DNA replication.
Delayed synthesis of virion structural proteins prevents

- When new viral DNAs are encapsidated they cannot be used as templates for additional DNA synthesis
- Consequently genome packaging into particles is delayed – transcriptional control
Inhibition of cellular DNA synthesis

- When viral DNA replication is carried out mostly by viral proteins, cell DNA synthesis is often inhibited
- Increases availability of substrates
- Adenovirus, herpesvirus, poxvirus
- Mechanisms not understood
Viral DNAs are synthesized in specialized intracellular
Mechanism of exponential viral DNA replication

Cellular retinoblastoma (rb) gene: Rb protein controls entry into S from G1
Loss is associated with tumors = tumor suppressor gene
Adenovirus E1A protein binds Rb
Limited replication of viral DNA

- Most DNA viruses: exponential replication of genomes
- Some establish long-term relationships with cells, number of genomes is limited
- Various mechanisms effect genome copy number
Parvovirus DNA Replication

Rep78/68 a site & strand-specific endonuclease
Parvovirus DNA replication

- DNA replicates only in cells coinfected with helper adenovirus
- Adenoviral helper proteins allow synthesis of large quantities of Rep 78/68
Parvovirus DNA replication

- When no helper adenovirus is present, Rep 78/68 level is low
- Little viral DNA synthesis occurs
- Genome integrates into host cell DNA
Papillomaviruses: Controlled and exponential replication from a single Ori