Papilloma and Polyomaviruses: Transformation and Cancer

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Papovaviruses

1. Classification: Polyoma, SV40, papilloma viruses
2. Historical perspective: SV40 as a probe for cellular functions
3. Host range: replication and “transformation”
4. Role of multifunction early viral proteins and transformation
5. Are papovaviruses human tumor viruses
Papovaviruses

Classification: Polyoma, SV40, papilloma viruses
Papovaviruses

Polyoma Viruses

- SV40 (primate)
- JC,BK (human)
- Py (mouse)
- MCPyV (human)
- ?

Papilloma

- Multiple species
- HPV 6,11 CD
- HPV 16,18 CIN
- HPV cutaneous
- *
<table>
<thead>
<tr>
<th>Feature</th>
<th>Polyoma virus</th>
<th>SV40</th>
<th>Papilloma virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>45 nm icosahedral</td>
<td>45 nm icosahedral</td>
<td>55 nm icosahedral</td>
</tr>
<tr>
<td>Symmetry</td>
<td>7 levo</td>
<td>7 dextro</td>
<td>7 levo (rabbit)</td>
</tr>
<tr>
<td>Triangulation number (+)</td>
<td>7 levo</td>
<td>7 dextro</td>
<td>7 dextro (human)</td>
</tr>
<tr>
<td>Number of structural subunits (60 × t)</td>
<td>420</td>
<td>420</td>
<td>420</td>
</tr>
<tr>
<td>Number of capsomers</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>DNA (m.w.)</td>
<td>3.4 × 10⁶</td>
<td>3.4 × 10⁶</td>
<td>5 × 10⁶</td>
</tr>
<tr>
<td>DNA content (% w/w)</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Virion (m.w.)</td>
<td>27 × 10⁶</td>
<td>27 × 10⁶</td>
<td>40 × 10⁶</td>
</tr>
<tr>
<td>Protein composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>major capsid protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(75% total protein)</td>
<td>47,000</td>
<td>45,000</td>
<td>54,000</td>
</tr>
<tr>
<td>minor capsid proteins</td>
<td>35,000</td>
<td>42,000</td>
<td>several</td>
</tr>
<tr>
<td>Histones (same composition in all viruses)</td>
<td>H3</td>
<td>15,300</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2A</td>
<td>14,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2B</td>
<td>13,800</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H4</td>
<td>11,300</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 5. Estimated prevalence of genital HPV infection among men and women between the ages of 15 and 49 years in the United States in 1987. (From ref. 113 with permission.)
Papovaviruses

Historical perspective: SV40 as a probe for cellular functions
Historical perspective

1. Risk of primary cell sources (AGMK)
2. Definition of specificity of restriction sites (Nobel Prize)
3. First sequenced genome
4. Deletion mutants and functional map (colinearity)
5. Probe for host functions
   a. Nuclear localization signal
   b. (Confirmed) splicing
   c. Identification of enhancer sequences (Py, SV40)
   d. Development of in vitro DNA replication
   e. co-IP –Rb, p53, others
6. Model of a DNA tumor virus
   a. Role of viral ts (tsA) mutants
Host Range: Replication and “Transformation”
Papovaviruses

Host range: replication and “transformation”:
SV40 permissive infection = lytic/monkey cell

SV40 non-permissive infection = transformation/mouse 3T3 cells
SV40 (semi-permissive) infection = lytic vs transformation/human diploid fibroblasts
Role of temperature-sensitive (ts) mutants

Papillomaviruses (HPV) and cell differentiation
A. SV40 (5243 bp)

- **Ori**
- **VP1**
- **VP2**
- **VP3**
- **L**
- **NTR (Regulatory Region)**
- **An**
- **Late Transcripts**
- **DNA**
- **Early Transcripts**

**ORF 1**
**ORF 2**
**ORF 4**
**ORF 5**
**Ori**
**NTR (regulatory region or enhancer)**
**P_R Early gene promoter**
**P_L Late gene promoter**
**Initiation codon**
**Termination codon**

Scale (bp): 0, 1000, 2000, 3000, 4000, 5000
Papovaviruses

Host range: replication and “transformation”:
SV40 permissive infection = lytic/monkey cell
FIG. 3. Replication cycle of polyoma and SV40. Steps in the replication cycle are indicated by numbers as follows: 1. adsorption of virions to the cell surface; 2. entry by endocytosis; 3. transport to the cell nucleus; 4. uncoating; 5. transcription of early region mRNAs; 6. translation of early proteins (T antigens); 7. viral DNA replication; 8. transcription of late region mRNAs; 9. translation of late proteins (viral proteins); 10. assembly of progeny virus particles.
Large T Protein

Amino acids

Polo

Ori binding

Zα

ATP binding

ATPase

p53 binding, Polo binding

X

Rb

DNA helicase activity

Functional domains of large T
DNA-Containing Viruses

Middle T antigen

Association of middle T with c-src and activation of c-src tyrosine kinase

Phosphorylation of middle T by c-src on Y

Transformation
Papovaviruses

Host range: replication and “transformation”:
- SV40 non-permissive infection = transformation/mouse 3T3 cells
  Integration of viral genome
- SV40 (semi-permissive) infection = lytic vs transformation/human diploid fibroblasts
  Role of temperature-sensitive (ts) mutants
SVtsA/3T3

37°C

confluent

33°C

Time in days

Cell Number

SV/3T3

37°C

confluent

33°C

Time in days
Papovaviruses
Papillomaviruses (HPV) and cell differentiation
Human Papillomavirus

Genome Organization and Protein Functions

Episomal Replication

URR rep. ori. enhancers

** E7

E6

Regulation

*** E4

**** E5a

Minor Capsid

E5a

L2

Major Capsid

L1

Reading Frames

1

2

3

7933/1 1000 2000 3000 4000 5000 6000 7000

- E6 binds p53 tumor susceptibility protein
- ** E7 binds retinoblastoma protein and liberates E2F
- *** E4 binds cytokeratin intermediate filaments
- **** E5a upregulates EGF receptor signal transduction

E1 and E2 bind to the replication origin
E2, E1 and E2 bind to the replication origin

regulate transcription
and replication
NORMAL AND HPV-INFECTED EPITHELium:

**KERATINOCYTE DIFFERENTIATION**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Event/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum corneum</td>
<td>Stratum corneum</td>
</tr>
<tr>
<td>Sloughing</td>
<td></td>
</tr>
<tr>
<td>Granular Layers</td>
<td></td>
</tr>
<tr>
<td>Nuclear degeneration, cornification</td>
<td></td>
</tr>
<tr>
<td>Filaggrin</td>
<td></td>
</tr>
<tr>
<td>Upper Spinous Layers</td>
<td></td>
</tr>
<tr>
<td>Cytokeratin filament aggregation</td>
<td></td>
</tr>
<tr>
<td>Keratins 1 &amp; 10 continue</td>
<td></td>
</tr>
<tr>
<td>Lower Spinous Layers</td>
<td></td>
</tr>
<tr>
<td>Keratins 1 &amp; 10</td>
<td></td>
</tr>
<tr>
<td>Transit Amplifying Cells</td>
<td></td>
</tr>
<tr>
<td>Keratins 1 &amp; 10</td>
<td></td>
</tr>
<tr>
<td>Frequent DNA replication</td>
<td></td>
</tr>
<tr>
<td>Basal Stem Cells</td>
<td></td>
</tr>
<tr>
<td>Sporadic DNA replication &amp; replenishment of transit cells</td>
<td></td>
</tr>
<tr>
<td>Keratins 5 &amp; 14</td>
<td></td>
</tr>
<tr>
<td>Basement Membrane</td>
<td></td>
</tr>
<tr>
<td>Dermis</td>
<td></td>
</tr>
<tr>
<td>Connective tissue (collagen), Fibroblasts, Lymphocytes, Vascular endothelium</td>
<td></td>
</tr>
</tbody>
</table>

**PAPILLOMAVIRUS REPRODUCTION**

<table>
<thead>
<tr>
<th>Event/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature virions released</td>
</tr>
<tr>
<td>Mature virions</td>
</tr>
<tr>
<td>Virion assembly</td>
</tr>
<tr>
<td>Late capsid proteins L2 and L1</td>
</tr>
<tr>
<td>Vegetative DNA amplification</td>
</tr>
<tr>
<td>High levels of early &amp; differentiation-dependent proteins</td>
</tr>
<tr>
<td>Koliocytosis</td>
</tr>
<tr>
<td>Differentiation-dependent E6 &amp; E7 proteins</td>
</tr>
<tr>
<td>Early proteins E1, E2, E5 &amp; E4</td>
</tr>
<tr>
<td>Possible alternative site of infection</td>
</tr>
<tr>
<td>Immediate early proteins E1, E2 &amp; E5</td>
</tr>
<tr>
<td>Primary infection</td>
</tr>
<tr>
<td>Establishment replication</td>
</tr>
<tr>
<td>Immediate early proteins E1, E2 &amp; E5</td>
</tr>
<tr>
<td>Epidermal Layer</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Keratin</td>
</tr>
<tr>
<td>Keratonyalin</td>
</tr>
<tr>
<td>Spiny</td>
</tr>
<tr>
<td>Germinal</td>
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</table>


Role of Multifunction Early Viral Proteins and Transformation
Papovaviruses

Role of multifunction early viral proteins and transformation:

Inactivation of cell growth suppressor
  What is pRb
  What is p53
Different strategies to a common end
SV40 early region (T,t), telomerase, (Sen6) and mutant ras (Hahn and Weinberg)
<table>
<thead>
<tr>
<th>Viral Protein</th>
<th>RB</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV40 LT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyoma LT</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Papilloma E7</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Papilloma E6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Adenoviruses E1A</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Adenoviruses E1B</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Proliferating cell cycle regulation (left) and HPV E7 protein over-ride of pRB in differentiated keratinocytes (right). The E2F:DP1 enhancer protein complex regulates genes necessary for deoxyribonucleoside triphosphate synthesis and replication proteins.
## Comparison of HPV

<table>
<thead>
<tr>
<th></th>
<th>low cervical cancer</th>
<th>high cervical cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>genome</td>
<td>6, 11</td>
<td>16, 18</td>
</tr>
<tr>
<td>p53 level</td>
<td>normal</td>
<td>low</td>
</tr>
<tr>
<td>ubiquitin E2</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>DNA status</td>
<td>episomal</td>
<td>integrated</td>
</tr>
<tr>
<td>telomerase</td>
<td>no</td>
<td>induced</td>
</tr>
</tbody>
</table>
Are Polyomaviruses (esp. SV40) DNA tumor viruses or Why is there still uncertainty about SV40 and mesotheliomas
Papovaviruses

Are papovaviruses human tumor viruses:

- HPV and cervical carcinogenesis (Zur Hausen)
- human anti HPV vaccine (Gardasil, Cervarix)
- SV40 and mesothelioma/other – controversy (Handout)
- Other?
  Feng et al. [Merkel Cell Carcinoma], Science 319:1096-1100 (Feb 22, 2008)
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