Polyomaviruses-- SV40

- 1st detected in 1953 in mice with leukemia
  - Extracts of mouse tissue contaminated with SV40 gave rise to salivary gland tumors in mice inoculated with tissue
  - Can induce tumors in many organs (as opposed to only a single organ of most viruses
  - “Transformation without replication”

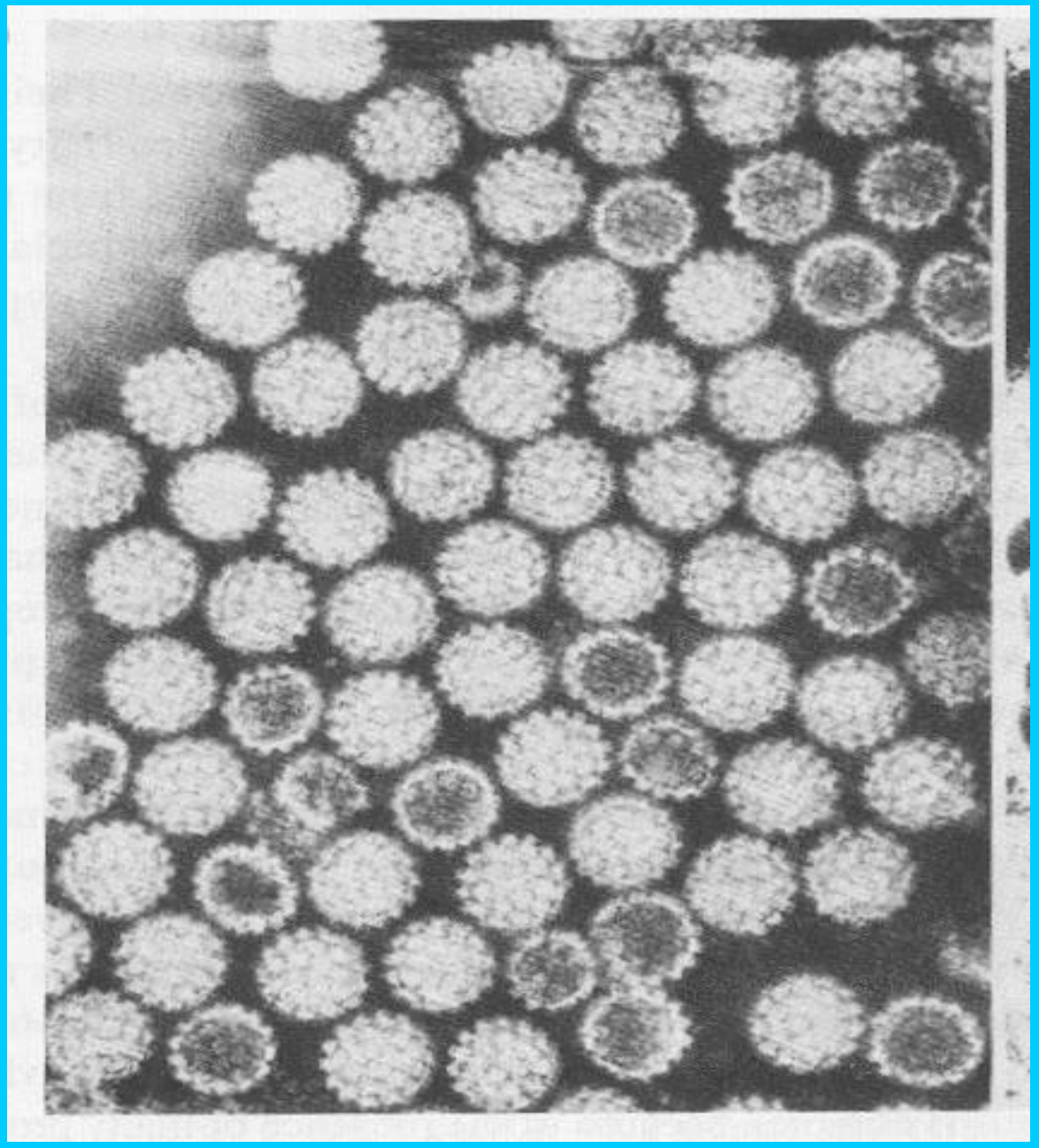
SV40

- The 40th virus isolate in cultured monkey kidney cells
  - Can be propagated only in B lymphocytes and transforms hamster fibroblasts (lymphotropic papovavirus [LPV])
  - 2 Human polyomalike viruses:
    - JC agent isolated in neurological disease
    - BK virus isolated in immunological dysfunction
      - These cross react serologically with SV40
      - Infection usually in childhood, but carried for life
    - In humans these are NOT oncogenic but can agglutinate RBCs

Structure of SV40

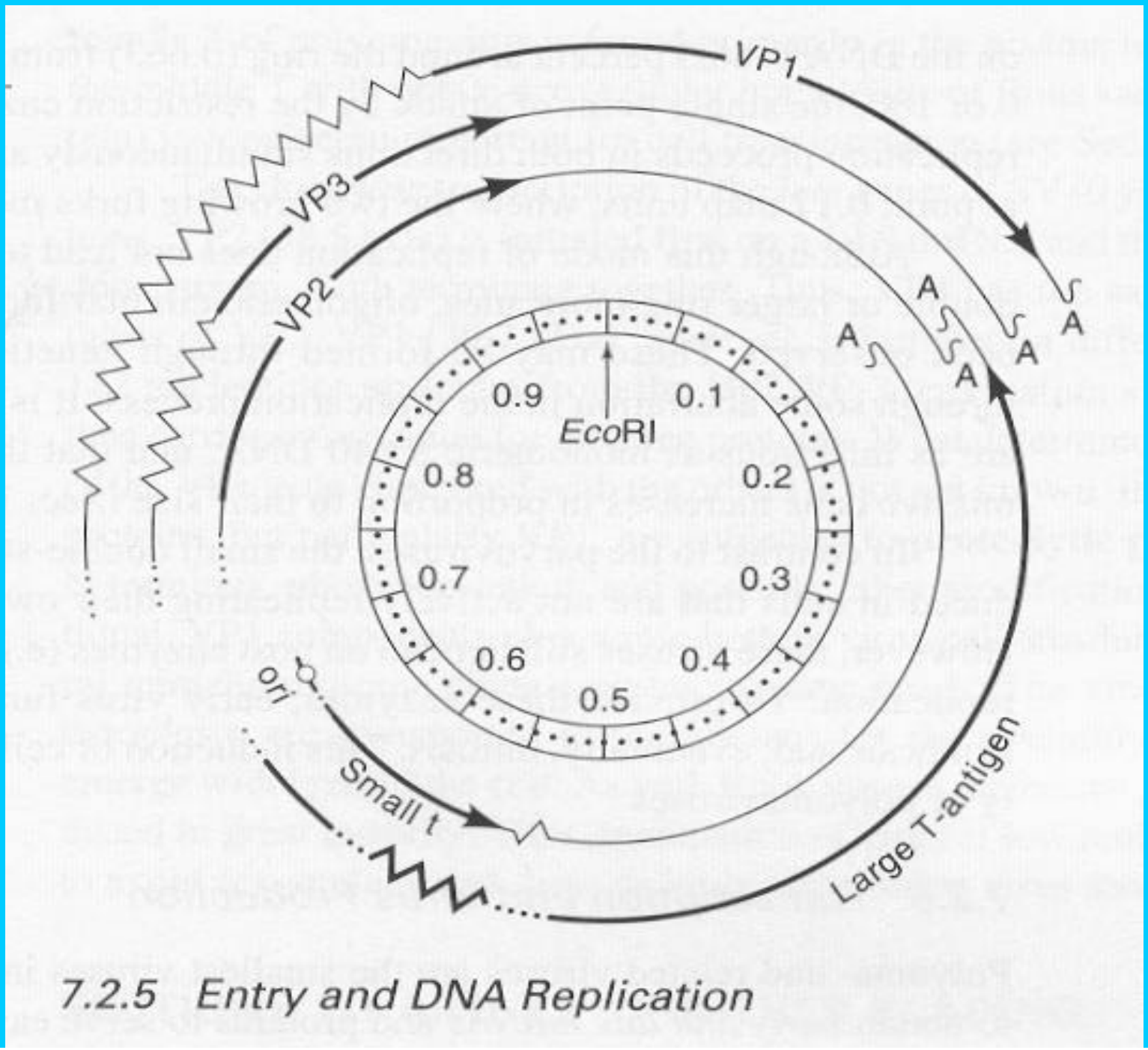
- Icosahedral with visible capsomers
- dsDNA, circular
- These can become integrated into host DNA
- Genetic information carried on both strands
  - DNA by itself is infectious
    - 3 coat proteins make up virion (VP1, VP2, VP3)
    - Associated with viral specific histone proteins

SV 40 Virus



Peptide map (gene regions)

- Complete sequence of DNA known
  - 2612 base pairs (570 is not transcribed, and is around point of origin of replication)
  - Two of the 3 proteins (VP2 and VP3) are encoded in the SAME reading frame on the same part of the genome
  - VP1 (about 75% of total virion protein) is read in a different reading frame



7.2.5 Entry and DNA Replication

Entry and DNA Replication

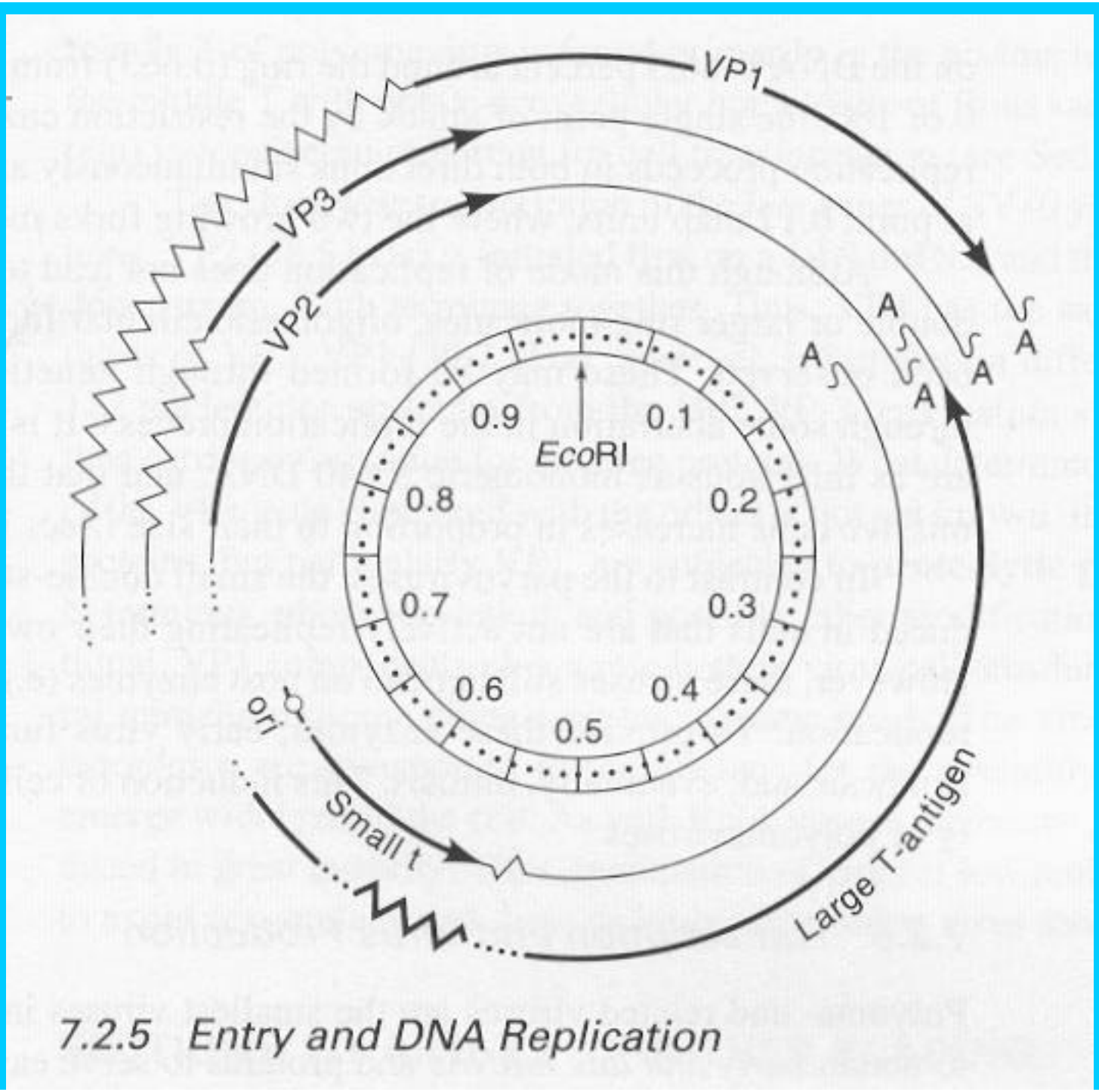
- Adsorbtion to cell surface and penetration by endocytosis
  - Site of entry in humans is probably respiratory tract, but unknown for sure
- Transported to nucleus, fuses with nuclear membrane, enters nucleus and uncoats
- Transcription of EARLY mRNA occurs with production of T antigens. (transforming Ags)
- Virus DNA replication occurs via LATE mRNAs and translation of late proteins gives rise to the progeny particles

Integration Process

- Replication of DNA requires prior transcription of early genes and formation of the T antigen that binds to the “origin” site on parental DNA
- Replication is bi-directional from single point of origin (**theta mode**)
  - Process requires: RNA primer synthesis, helix unwinding, formation of DNA nascent strands (Okazaki fragments), primer degradation, gap filling, ligase acivity, and the action of topoisomerase II (enzyme that supercoils DNA
- Entire replication process, which occurs in nucleus of cell, takes about 5 minutes

DNA Replication

- There is **NO** concatemer formation
- Viruses can be replicated in cells that are not dividing themselves
  - Viruses depend on host enzymes (DNA polymerase, …) and virus is able to stimulate these host proteins by some of the early proteins made (i.e., T antigens)



7.2.5 Entry and DNA Replication

Transcription & Virus Production

- Single cycle for SV40 is 48-72 hours
- EARLY transcripts
  - mRNAs represent transcripts from near the DNA replication origin (0.66 to 0.17) in a counter-clockwise direction.
  - These transcripts have different spliced-out regions
- LATE transcripts
  - Transcribed in clockwise manner from other ½ of DNA circle, starting at about 0.73 and going to 0.16
  - Transcription is on the strand complementary to the strand copied for early mRNAs (5' →3')

EARLY TRANSCRIPTS

- Small t
  - 20 kDa (small excision of DNA bases) and terminates at stop codon ~ 522 nucleotides downstream
- Large T
  - 90-100 kDa (has larger excision of ~ 400 bases that removes termination codon of small t)
  - Large T and small t share first 100 amino acids

Tumor (T and t) Proteins

- Large T synthesized in cytoplasm and moves into nucleus and functions in viral DNA replication (induces synthesis of proteins leading to cell division)
- Small t found between nucleus and cytoplasm and is associated with the accumulation of viral DNA that interacts with cellular proteins

Late gene transcription

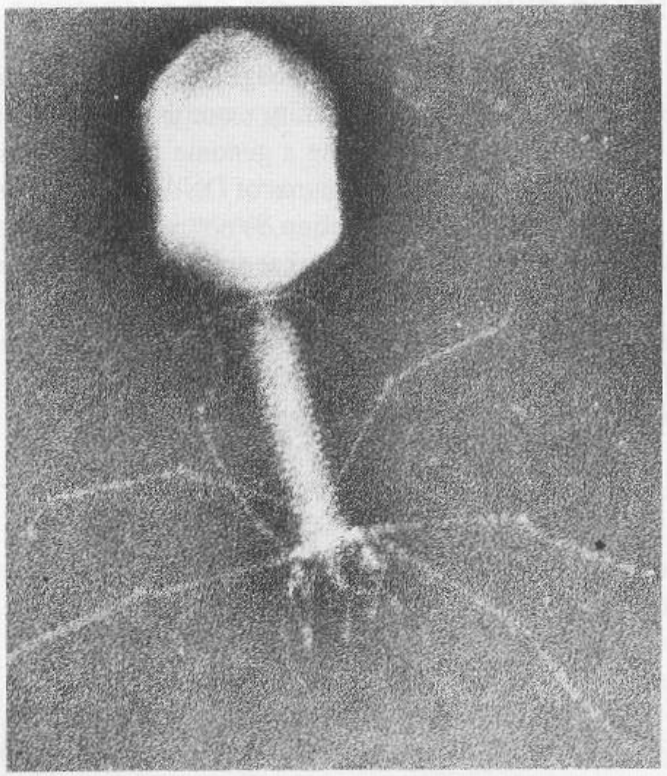
- Clockwise transcription yields 3 coat proteins
  - VP2 initiated 1st and then VP3 118 codons downstream. VP3 has same sequence as last 2/3 of VP2
  - Both terminate together
  - VP1 initiated 122 nucleotides upstream from VP2-VP3 termination site
- Nucleotide sequence carries information for all 3 proteins
- Proteins made in cytoplasm are all carried back to the nucleus for assembly

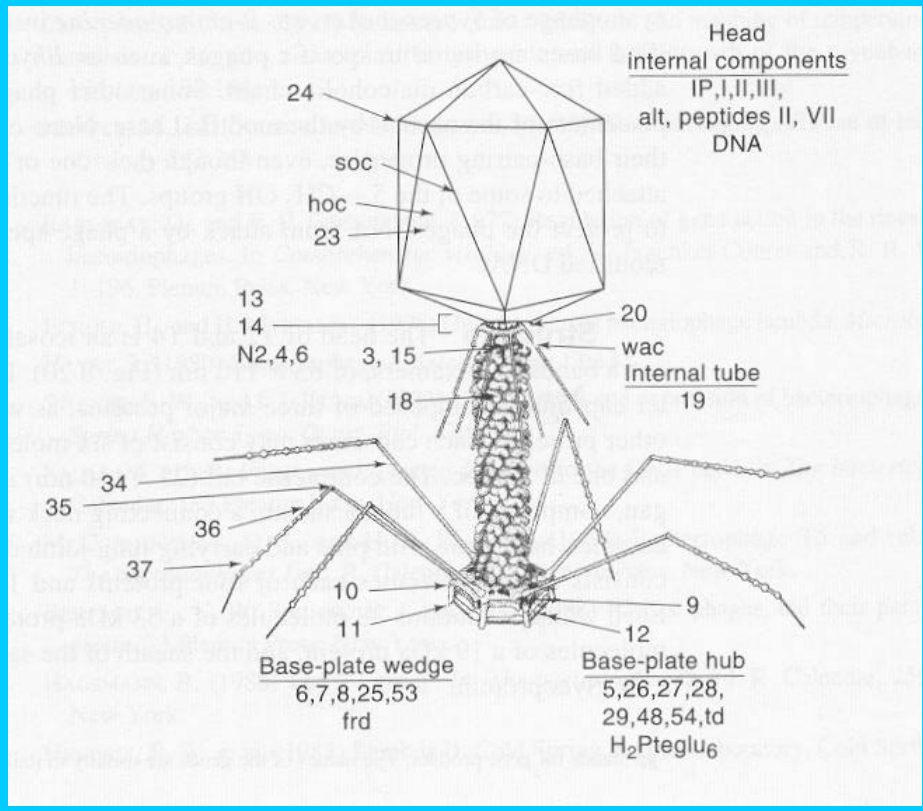
TABLE 10.3

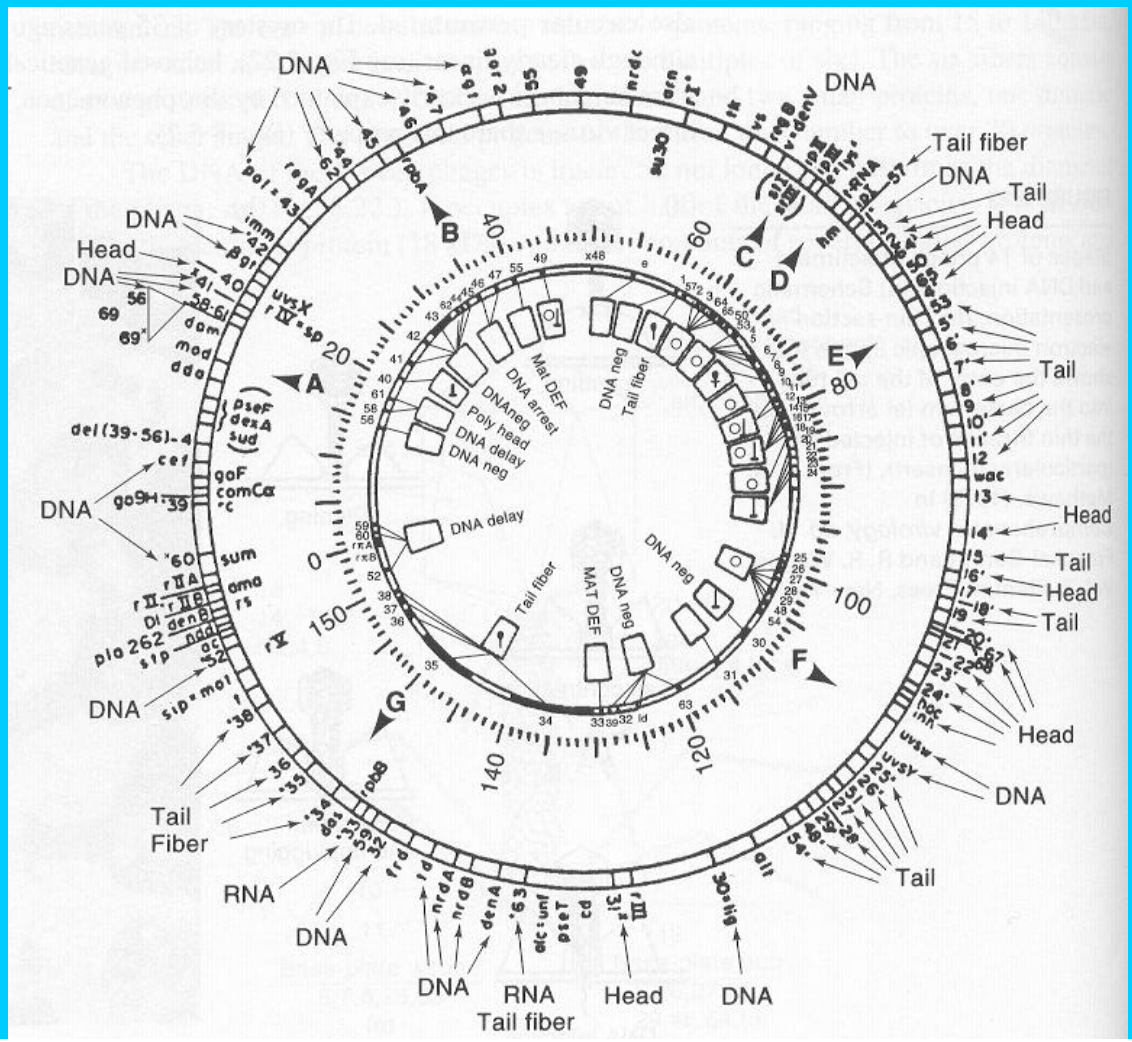
Properties of the SV40 large T antigen

1. Product of the A gene
2. A phosphoprotein of 100 kDa
3. Found primarily in the cell nucleus
4. Stimulates host cell DNA synthesis
5. Binds to SV40 DNA at origin of replication
6. Essential for initiation of viral DNA synthesis
7. Essential for establishment and maintenance of transformation
8. Has ATPase activity associated with a helicase (i.e., unwinds DNA)
9. Plays a role in virus replication; its expression is necessary for activation of virus production in nonpermissive cells
10. Facilitates replication of human adenovirus in monkey cells
11. Induces ribosomal RNA synthesis
12. Binds to p53 and RB suppressor gene products
13. Binds DNA polymerase α
14. Binds cellular heat shock protein, hsp70

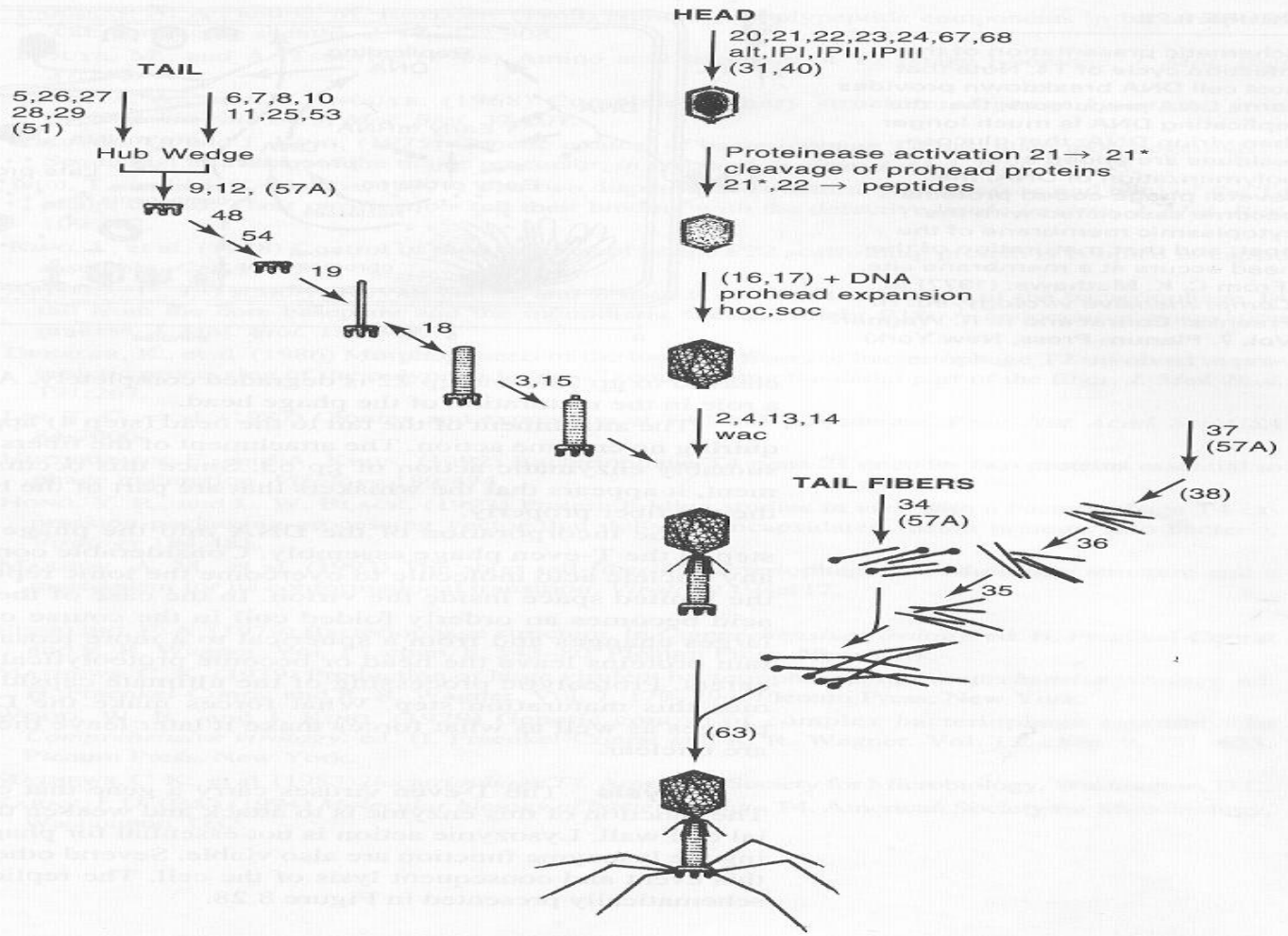
Complex Viruses: Bacteriophages







Class	Gene	Function
A	34–38, 57, 63	Tail fiber and tail fiber joining to tail plate
B	5–12, 25–29, 48, 51, 53, 54	Tail plate and tail plate assembly
C	3, 18, 19	Tail core and sheath
D	2, 4, 13–15, 50, 64, 65, *wac*	Collar, head–tail joining, head completion
E	16, 17, 20–24, 31, 40, 49, *hoc, soc*	Head and head filling



1. Assembly of the base plate, leading to
2. Assembly of the tail tube and sheath
3. A separately initiated assembly of the head
4. An attachment of tail to head, and finally
5. An attachment of the separately assembled tail fibers to that particle.

FIGURE 8.28

Schematic presentation of the infection cycle of T4. Note that host cell DNA breakdown provides some DNA precursors; that the replicating DNA is much longer than virion DNA; that glucose residues are added after polymerization of DNA; that several phage-coded proteins become associated with the cytoplasmic membrane of the host; and that maturation of the head occurs at a membrane site. (From C. K. Mathews. (1977) In *Comprehensive virology*, ed. H. Fraenkel-Conrat and R. R. Wagner. Vol. 7. Plenum Press, New York)