Viral Assembly

Assembly of TMV (Helical Virus)

- Consists of a single + sense ssRNA
- Embedded in framework of small identical protein molecules (“A” protein)
- Isolated protein can be polymerized into a helical structure without any RNA component (helical structure is a property of protein)

- TMV protein structure composed of discs
- Each disc contain 17 subunits/ring

- Assembly may involve discs being added to growing helix→ converted to a "lock-washer form"→ RNA trapped inside groove→ successive discs added

TMV "Travelling Loop" model of assembly

- Hairpin structure of TMV RNA packaging site attaches to disc in central core
  - The nucleotides in the ds stem then unpair and more of the RNA is bound within the groove and more discs enter into lock-washer configuration

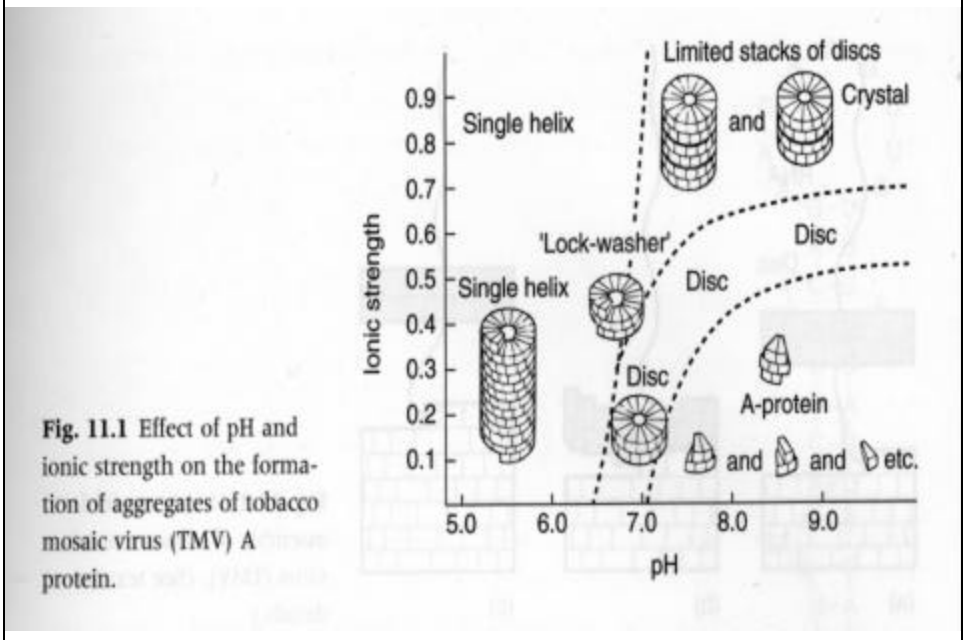

Fig. 11.1 Effect of pH and ionic strength on the formation of aggregates of tobacco mosaic virus (TMV) A protein.

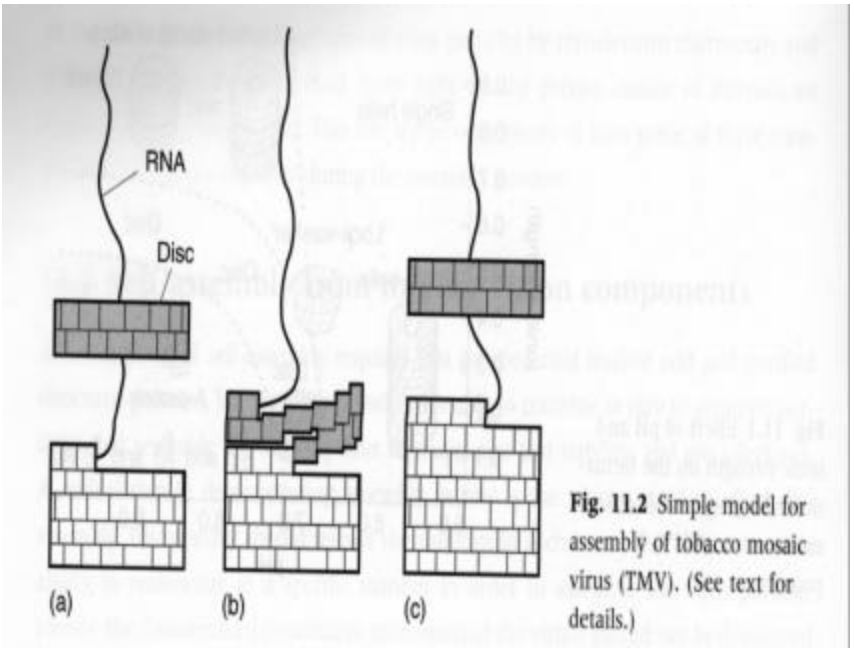

Fig. 11.2 Simple model for assembly of tobacco mosaic virus (TMV). (See text for details.)

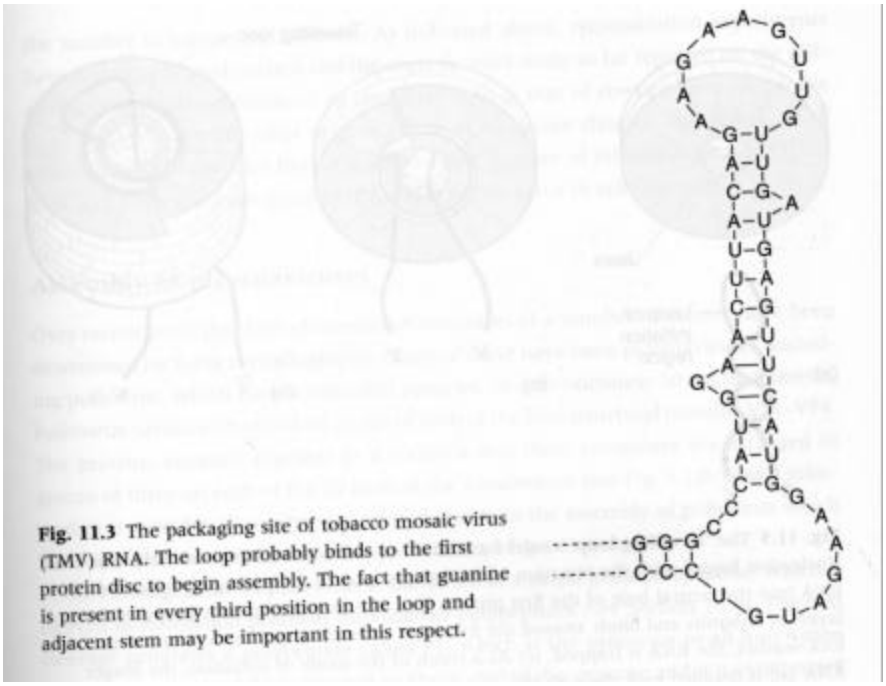

Fig. 11.3 The packaging site of tobacco mosaic virus (TMV) RNA. The loop probably binds to the first protein disc to begin assembly. The fact that guanine is present in every third position in the loop and adjacent stem may be important in this respect.

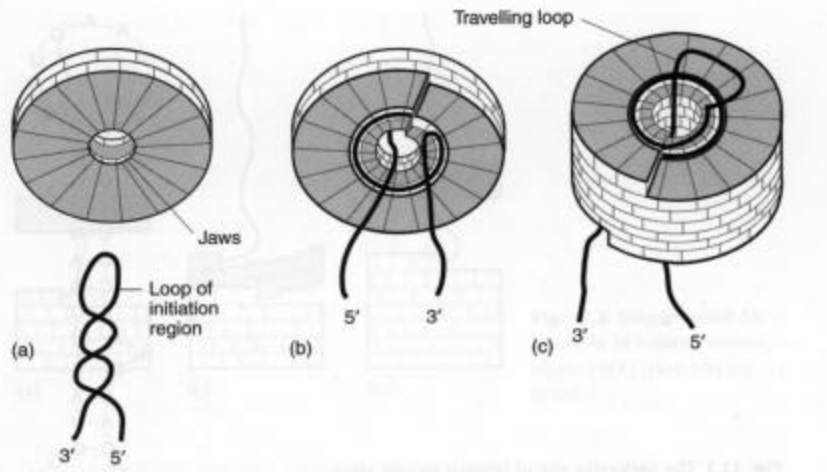

Fig. 11.5 The 'travelling loop' model for tobacco mosaic virus (TMV) assembly. (a) Nucleation begins with the insertion of the hairpin loop of the packaging region of TMV RNA into the central hole of the first protein disc. The loop intercalates between the two layers of subunits and binds around the first turn of the disc. (b) On conversion to the lock-washer, the RNA is trapped. (c) As a result of the mode of initiation, the longer RNA tail is doubled back through the central hole of the rod, forming a travelling loop to which additional discs are added rapidly.

Assembly of Picornaviruses

- Icosahedral structures
- Consists of 60 copies of each of the 4 structural proteins (VP1, VP2, VP3 and VP4)
  - These associate together in a complex and complexes are arranged in groups of three on each of the 20 faces

- Entire genome copied as a single polypeptide
  - This is then cleaved into smaller peptides
  - First cleavage product is "P1" = precursor to all the other virion coat proteins
  - Cleavage of P1 gives rise to VP0, VP1 and VP3
    - These 3 proteins form a complex = 5S
    - Five of the 5S complexes come together to form a 14 S pentamer subunit
    - 12 of the 14S complexes aggregate to form an empty 73S capsid
    - The + sense RNA is added (VPg protein at the 5' end of RNA may be involved in recognition of RNA with capsid proteins)
    - VP0 is cleaved → VP2 and VP4
    - RNA added after completion of capsid, not like TMV

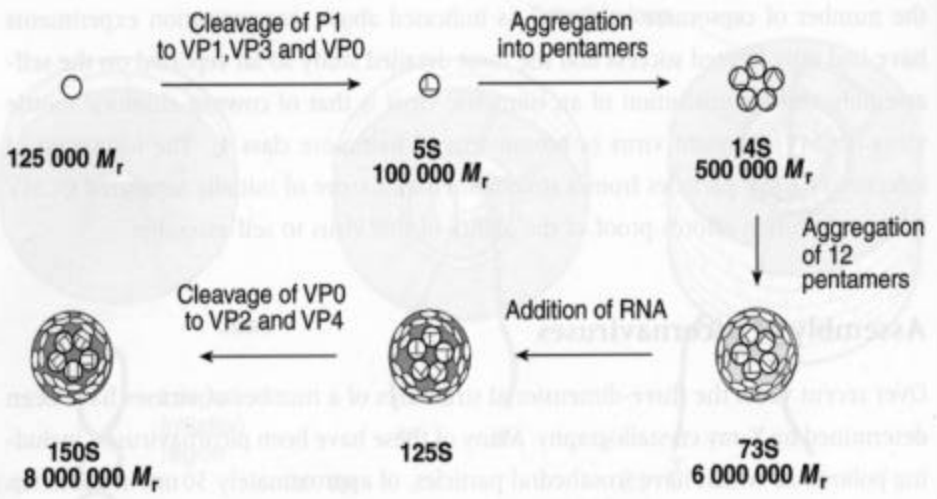

Fig. 11.6 Summary of the steps involved in the assembly of poliovirus.

Adenovirus Assembly

- Contains at least 10 different proteins
- The icsoahedral structure consists of 240 proteins that are on external face surface and have 6 fold symmetry (hexon capsomeres) and 12 are arranged at the verticies of the icosahedron with 5 fold symmetry (total of 252 capsomeres)
- Fiber structures project out from the vertices (pentons)

- Each of the individual components are assembled independently of each other and are brought together in a directed manner
- Assembly takes place in the nucleus, the proteins are made in the cytoplasm
- There is a large protein ("scaffolding protein") that is necessary for assembly but is not present in the final capsid

Adenovirus Assembly

- Takes place in the nucleus
- DNA replication occurs in nucleus
- Proteins must be translocated there at early stage
- Proteins that form the fibers, the base of the penton capsomere and the hexon capsomere are made independently from each other in the cytoplasm

- The fiber and hexon proteins come together independently to form trimer intermediates (the hexon trimer formation requires a protein of ~ 100,000 Mr that interacts with hexon monomers- this is NOT found in the final structure= **scaffolding** protein)
- The penton monomers form a penton base
  - Fiber trimers and penton base form a penton capsomere

- Scaffolding proteins are removed by proteases, the hexon subunits come together in an icosahedral structure, and then core DNA is added.
- Protease in the particle cleaves various components to create an infectious particle

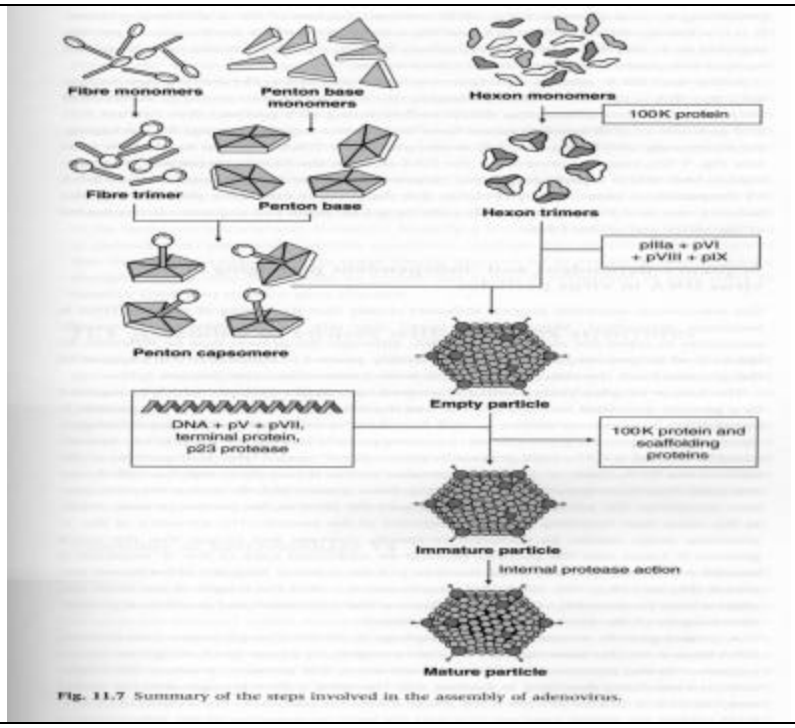

Fig. 11.7 Summary of the steps involved in the assembly of adenovirus.

Assembly of complex Viruses

- Use of conditional lethal (unable to produce infectious particles when bacteria grown under non-permissive conditions) mutants of T4 (bacteriophage) to understand processes leading to complex virus formation
  - These can produce some proteins but not others and some completed particles showing that the processes are not intimately linked with each other

- Found that morphogenesis of phage T4 from partially assembled components could be made *in vitro*
  - Different mutant components could be mixed to make infectious particles
- Next figure shows sequence of head formation

- Several proteins come together to form an immature prohead which contains all the elements of icosahedral symmetry
- Head then undergoes maturation process and it acquires the genome DNA with other additional proteins and loses scaffolding proteins (use of pulse-chase experiments to show stages)

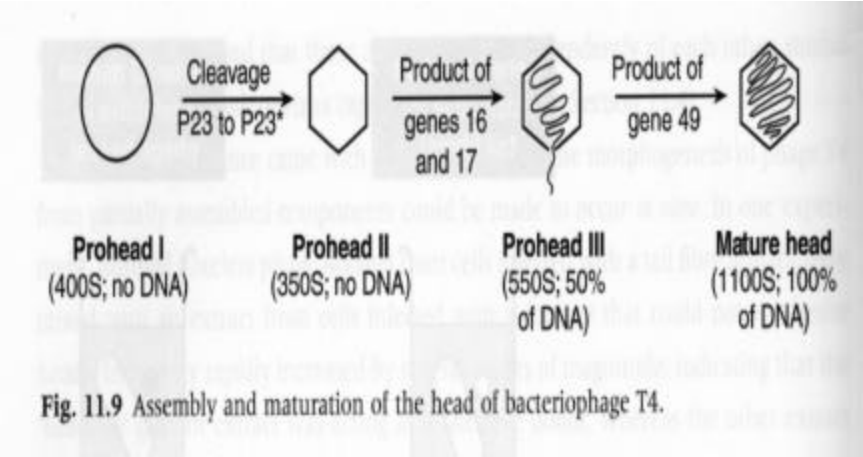

Fig. 11.9 Assembly and maturation of the head of bacteriophage T4.

- The DNA of T4 is circularly permutated
  - The immature phage head cuts a defined length of DNA from the concatemeric replicative intermediate
  - The size of the head determines the length of the encapsidated DNA

Assembly of T4 tail & tail fibers

- Scaffolding proteins involved and each component must be added in correct sequence
- After completion of all individual components, the heads spontaneously join with the base plate (requires active participation of gene product 63)
  - Not understood why a head with 5 fold symmetry complexes stably with a tail with 6 fold symmetry?

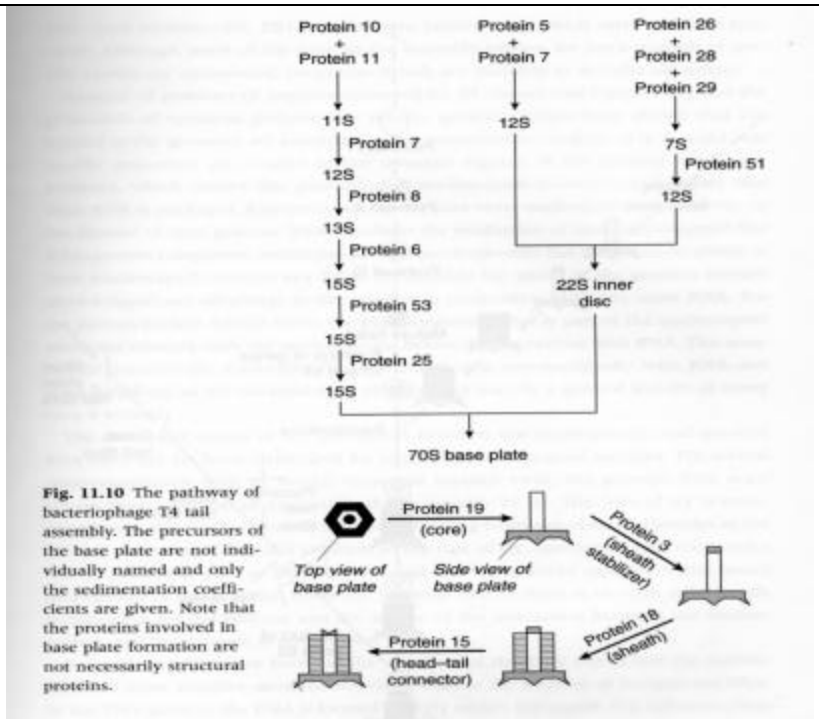

Fig. 11.10 The pathway of bacteriophage T4 tail assembly. The precursors of the base plate are not individually named and only the sedimentation coefficients are given. Note that the proteins involved in base plate formation are not necessarily structural proteins.

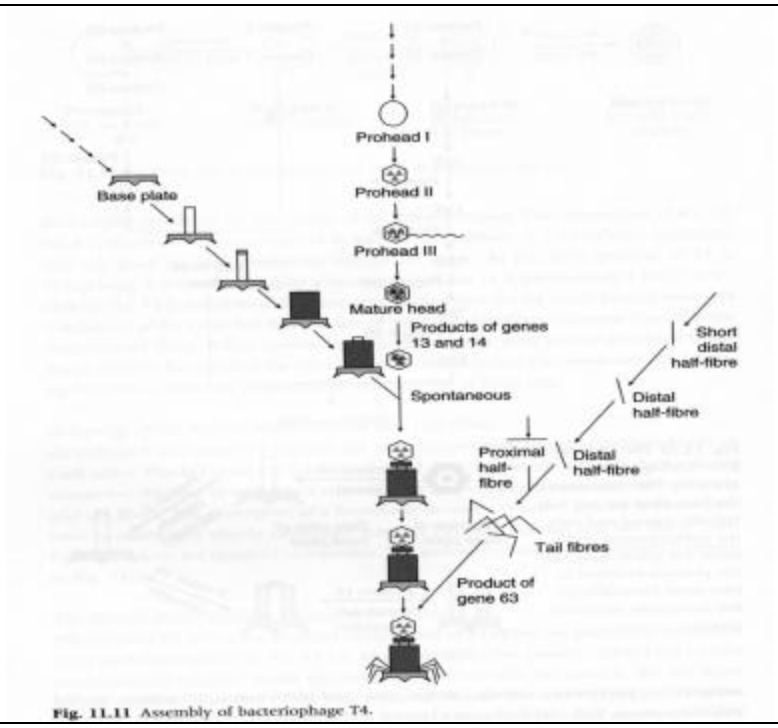

Fig. 11.11 Assembly of bacteriophage T4.

Acquisition of lipid envelope

- Most viruses acquire envelope from “budding” process
  - Nucleocapsids form in cytoplasm
  - Viral glycoproteins (transmembrane) accumulate in patches on plasma membrane
  - Cytoplasmic tails of glycoproteins that protrude from membrane attach to nucleocapsid
  - Virion is formed by budding

- Other viruses may bud into the endoplasmic reticulum and then are released to the exterior from the golgi complex

- Herpes virus is made in the nucleus
  - May bud through inner membrane and exits through the ER (connected to the inner membrane)
  - Or– buds through inner membrane and then fuses with outer membrane (losing membrane) and then buds through plasma membrane