Nermut/Steven (eds) Animal Virus Structure

CHAPTER 20

Papovaviridae

TIMOTHY S. BAKER[1] and IVAN RAYMENT[2]

[1] *Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A. and*
[2] *Department of Biochemistry, University of Arizona, Tucson, AZ 85721, U.S.A.*

Family: Papovaviridae
Genus: Papillomavirus. Species: rabbit (Shope) papilloma virus; also cow, deer, dog, goat, hamster, human and sheep papilloma virus
Genus: Polyomavirus. Species: polyoma virus (mouse); also BKV and JCV (human), HD (stump-tailed macaque), K (mouse), RKV (rabbit) and SV40 (monkey)
The prefix 'papova' derives from pa(pilloma), po(lyoma) and va(cuolating agent), the original name for Simian Virus 40.

20.1. General characteristics

Papovaviruses are non-enveloped isometric particles ranging in size from ≈45 nm (polyomaviruses) to 55 nm diameter (papillomaviruses). They have a protein shell (capsid) composed of 72 capsomers in a skew icosahedral arrangement which encapsulates a 'minichromosome' composed of a single molecule of closed circular double-stranded DNA associated with cellular histones: there are no lipids present. Non-infectious polymorphic forms, including empty capsids, small isometric particles and hollow cylindrical aggregates of capsomers, are usually present in virus preparations. Their role in viral replication is unclear; they may be assembly intermediates, although they most likely represent either byproducts of abortive assembly pathways or artifacts of isolation procedures.

Papovaviruses replicate and assemble in the nuclei of infected cells, are resistant

to treatment with acid, heat or ether, and most species are potentially oncogenic. Papillomaviruses cause tumors in natural hosts, whereas polyomaviruses cause tumors in species different from the species of origin. Of all transforming viruses known, polyomaviruses contain the smallest amount of genetic information. Several species haemagglutinate by reacting with neuraminidase-sensitive receptors.

Polyoma and SV40 are the most extensively characterized papovaviruses, in part due to the absence of a reproducible cell culture system permissive for replication of the papillomaviruses. Polyoma virus is described in detail, since it is the first animal virus whose capsid structure was solved at low-resolution by X-ray crystallography. Its structure is of particular interest because the arrangement of subunits in the polyoma capsid violates a fundamental prediction of the widely accepted dogma concerning the construction of icosahedral virus shells (Caspar and Klug, 1962).

The papovaviruses show remarkable similarities in their structural and genetic organization, so that polyoma virus can be considered to be representative of the entire class. Fig. 20.1 illustrates the distinctive surface morphologies of polyoma and SV40 viruses revealed by electron microscopy.

20.2. Polyoma virus (Py): physical and chemical characteristics

Mature virions are isometric particles (Fig. 20.1) with a diameter of 49.5 nm (interparticle distance in crystals) and a mass of about 25×10^6 daltons. They sediment at 250 S and have a buoyant density of 1.34 g/cm^3 in CsCl. Each particle contains a nucleohistone complex contained within a shelf of protein subunits (the capsid). Table 20.1 compares several chemical and physical properties of Py, SV40 and the papilloma viruses.

The genome consists of a single molecule of closed circular double-stranded DNA (M_r 3.5×10^6; 5292 basepairs; 47.3% GC content). Isolated DNA contains approx. 25 right-handed superhelical turns, is infectious, and codes for three structural (VP1, VP2, VP3) and three nonstructural proteins (small, middle and large T antigens). In the virion, the genome is associated with an approximately equal mass of cellular histones (H2A, H2B, H3 and H4) forming a 'minichromosome' with $\approx$25 nucleosomes (Fig. 20.2). During the lytic cycle the 'minichromosomes' are used as templates for DNA replication or for the transcription of 'early' and 'late' genes.

20.3. Architecture of the virion

Icosahedral capsid

The structure of the virus capsid has been extensively studied by electron microscopy (Finch and Crawford, 1975; and Fig. 20.1) and by X-ray crystallography

TABLE 20.1.

Chemical and physical properties of Papovaviruses

	Py	SV40	Papilloma
Diameter (nm)	45 (EM) 49.5 (XR)	45 (EM)	50–55 (EM)
Symmetry	Icosahedral	Icosahedral	Icosahedral
Triangulation number	7d	7d	7d (human) 7 I (rabbit)
Number of capsomers	72	72	72
Number of VP1 molecules	360	360 (?)	360 (?)
Number of VP2 and VP3 molecules	≈60	≈60	>60[a]
Virion mass (daltons)	24.6×10^6	27×10^6	40×10^6
Sedimentation coefficient (S)			
virion	240	240	296–300
capsid	180	180	168–172
Density in CsCl (g/cm³)			
virion	1.34	1.34	1.34
capsid	1.29	1.29	1.29
Protein composition (M_r)			
VP1 (structural viral proteins)	42404	40168	57000 (human)
VP2	34761	38351	(≈10 minor proteins)
VP3	22866	26965	
H3 (cellular histones)	15300	15300	15300
H2A	14000	14000	14000
H2B	13800	13800	13800
H4	11300	11300	11300
DNA molecular weight	3.5×10^6	3.5×10^6	$4.5–5.5 \times 10^6$
Number of basepairs	5292 (A2)	5243	≈7500
% GC	47.3	40.7	41–50
DNA content (%w/w)	14	13	12.5
Number of superhelical twists	20–30	22–26	?
Non-structural viral proteins			
Large T antigen	87991	81632	?
Middle T antigen	49710	not found	?
Small T antigen	22785	20451	?

Abbreviations: EM, electron microscopy; XR, X-ray diffraction; GC, guanine-cytosine content; CsCl, cesium chloride; VP, viral protein; A2, large plaque, A2 strain.

The molecular weights for Py and SV40 viral proteins are calculated from the DNA sequences.

Data compiled from the suggested reading list.

[a] Including other minor proteins.

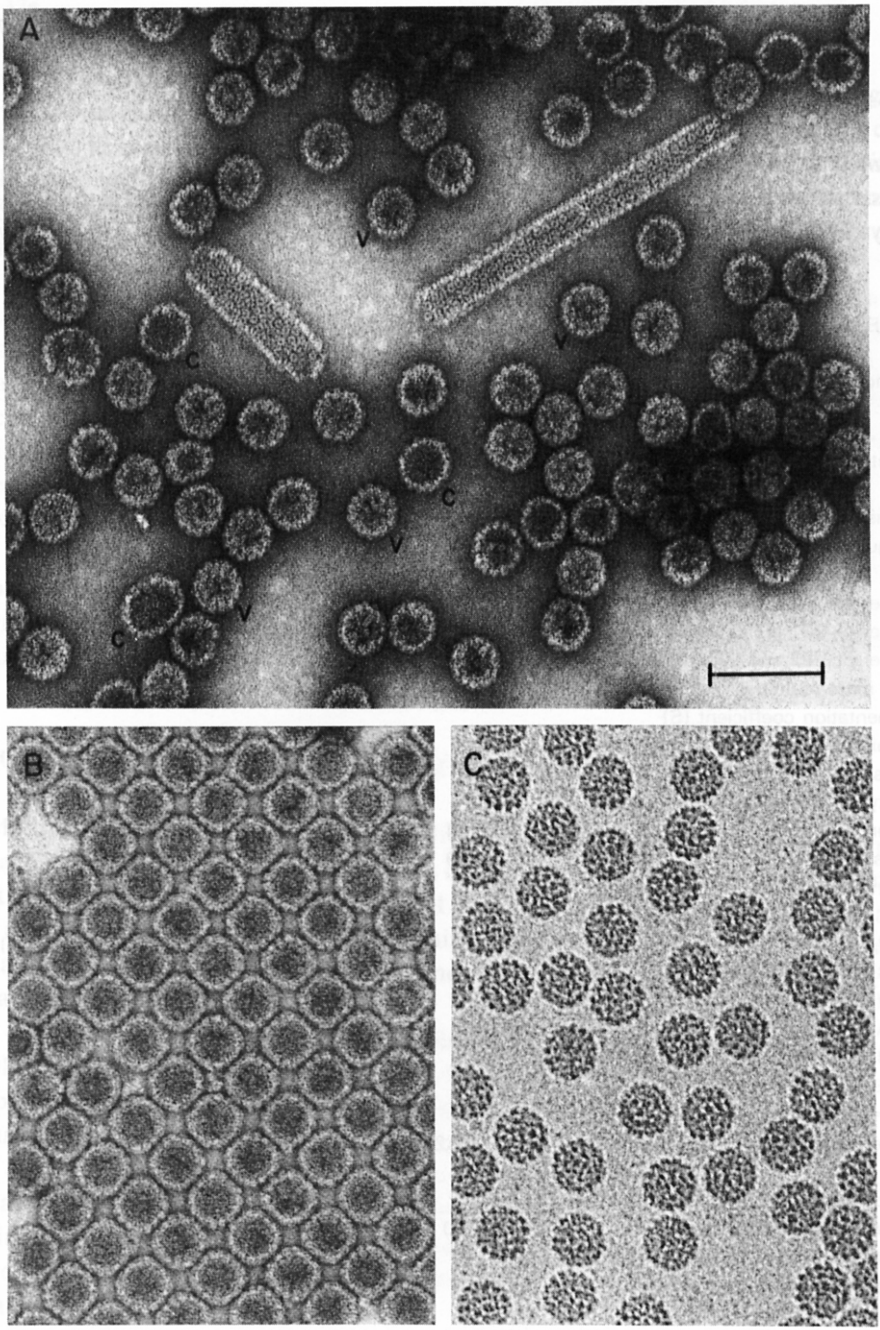


Fig. 20.1. (A) Negatively stained Py virions (V), capsids (C) and wide tubes. (B) Negatively stained SV40 array prepared by the method of Horne and Pasquali-Ronchetti (1974). (C) SV40 suspended in vitreous ice ($\approx -170°C$) over a hole in the carbon substrate. The unstained virus appears black because it is denser than ice. All images are at the same magnification (scale marker in (A) = 0.1 μm). (B) and (C), which illustrate the morphological comparison between SV40 and Py, were obtained as part of a collaborative study (Baker, Drak and Bina (1985) Biophys. J. 47, 50a).

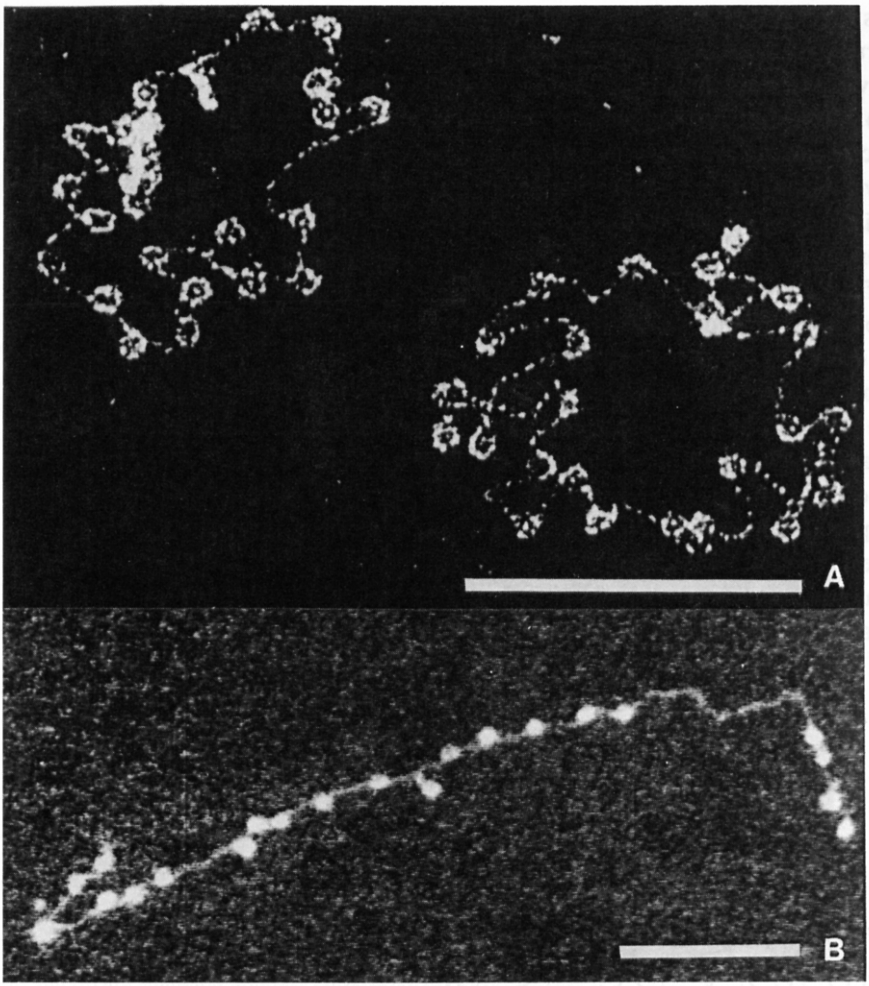


Fig. 20.2. (A) Dark-field electron micrograph of SV40 'minichromosomes' displaying the organization of nucleosomes on the circular, double stranded DNA molecule (reproduced by permission of S. Saragosti, G. Moyne and M. Yaniv). Bar = 100 nm. (B) Dark-field, scanning transmission electron micrograph of an unstained, freeze-dried SV40 'minichromosome' which has opened and spread out on the grid, displaying the characteristic 'beads-on-a-string' appearance of the nucleosomes on the DNA (kindly provided by J. Wall, J. Wooley and A. Varshavsky). Bar = 100 nm.

(Rayment et al., 1982). The capsid is composed of 72 morphological units (capsomers) arranged on the vertices of a $T = 7$d (right-handed) icosahedral surface lattice. There are 12 pentavalent capsomers (each adjacent to five hexavalent capsomers) located at the twelve icosahedral vertices, and 60 hexavalent capsomers (each adjacent to one pentavalent and five hexavalent neighboring capsomers) located in groups of three on each of the 20 icosahedral faces (Fig. 20.3).

Figs. 20.4 and 5 summarize the X-ray crystallographic studies of the empty capsid structure. All 72 capsomers are pentamers of VP1. This arrangement of subunits violates the theory of quasi-equivalence set forward by Caspar and Klug (1962) to explain how a virus shell could be constructed from a large number of identical subunits in such a way as to conserve subunit–subunit interactions. This theory predicts that a $T = 7$ capsid should contain 420 ($= 7 \times 60$) structural subunits in 72 capsomers, of which 12 are pentamers and 60 are hexamers.

There are six subunits (seven were predicted) in each icosahedral asymmetric unit, five from the hexavalent and one from the pentavalent pentamer, and they each occupy a symmetrically distinct environment. These six units can be put into three different classes according to their bonding relations inferred from the electron-density map (schematically illustrated in Fig. 20.5e,f).

The electron-density map may be divided into two regions: the protruding pentagonal caps (Fig. 20.5a,b) and the basal parts connecting capsomers (Fig. 20.5c,d). The contacts between capsomers extend inward from ≈ 21 nm radius of the capsid. The protruding caps are ≈ 8.5 nm in diameter and extend out ≈ 4 nm. The central hole in each capsomer has a diameter of 4 nm at a radius of 21 nm and tapers shut 1.5 nm from the surface, leaving a dimple on the top of the cap. The basal parts of the capsomers extend inwards ≈ 4 nm. The map suggests a two-

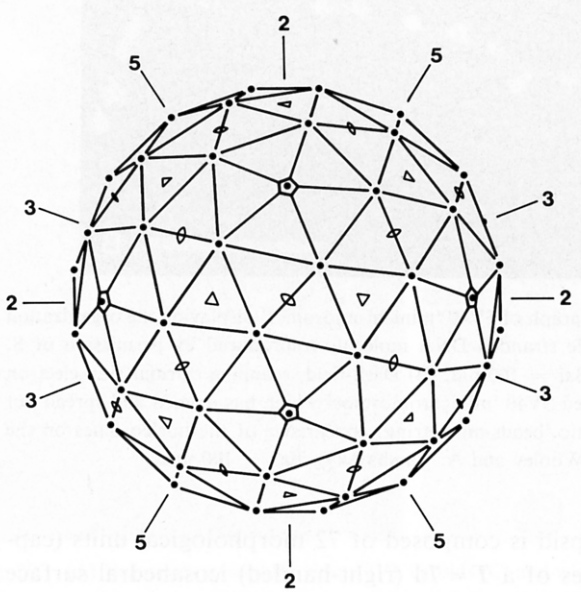


Fig. 20.3. $T = 7$d icosahedral surface lattice with five-, three- and two-fold axes marked ◇, △ and () respectively. The drawing shows one side of the polyhedral surface, which consists of 60 six-coordinated and 12 five-coordinated lattice points at the same radius. The location of the six-coordinated point is that determined for the hexavalent morphological unit in the polyoma capsid.

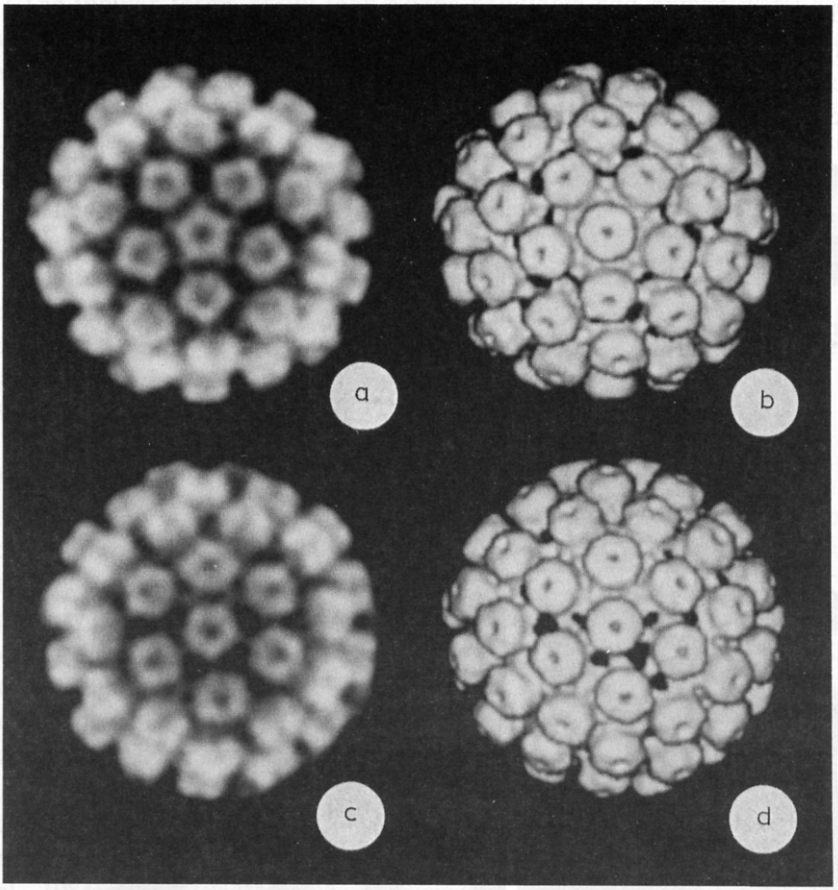
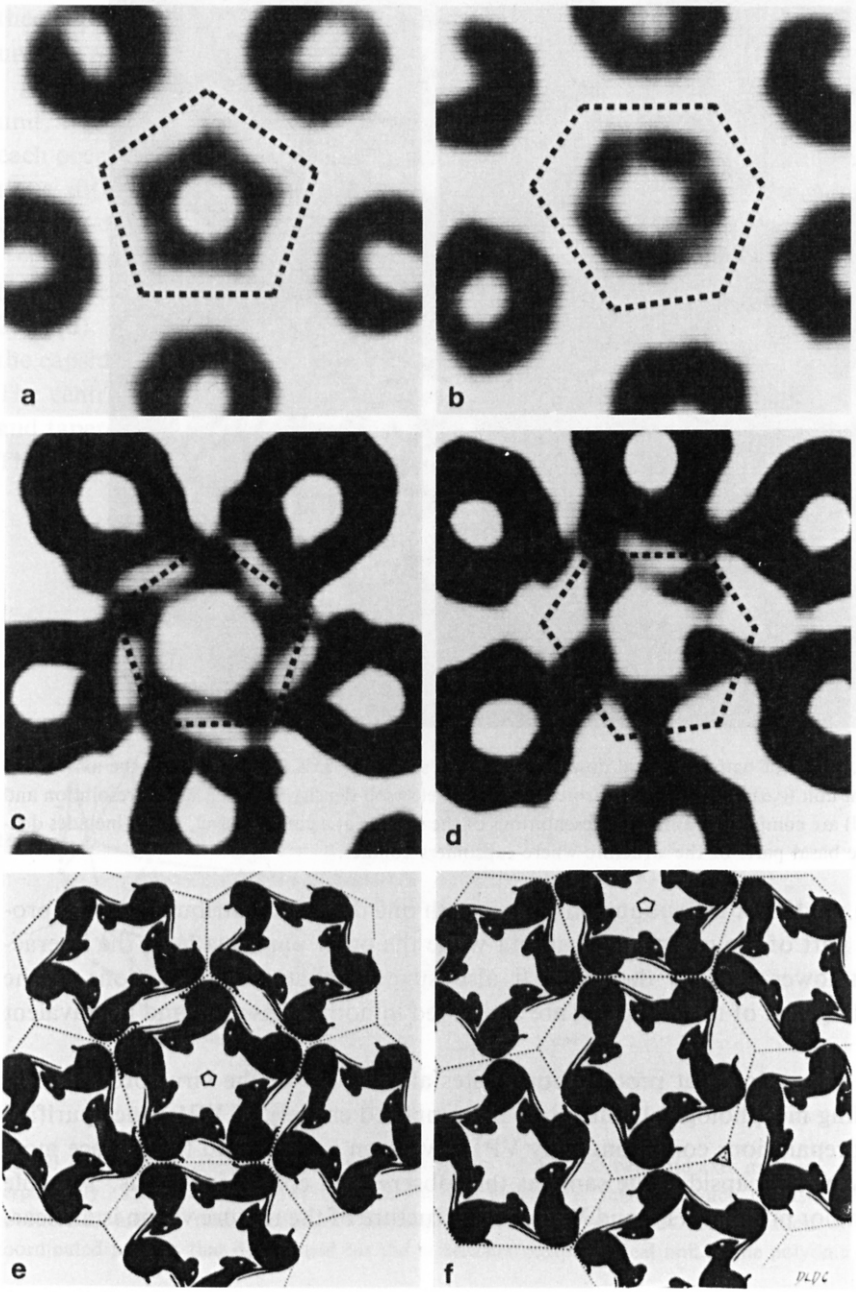


Fig. 20.4. Views of half the capsid down the five-fold symmetry axis (a,b) and down the axis of the hexavalent unit (c,d). (a) and (c) are projections of the electron-density map at 2.25 nm resolution and (b) and (d) are computer graphics representations of the surface at a contour level, which includes density in the basal parts of the structure where capsomers contact.

domain model for the subunit structure, with one domain contributing to the protruding part of the morphological unit while the other participates in the interactions at lower radii in the shell. It also suggests that the interactions in the protruding part of the capsomer are conserved in both hexavalent and pentavalent units.

VP1, the major coat protein, constitutes about 80% of the virus protein. The protruding morphological units must be composed entirely of VP1, since purified capsid preparations containing only VP1 have been isolated and the surface morphology of the capsid is the same as that observed in complete virions. The role of the minor proteins VP2 and VP3 in the structure of the mature virion is unclear.

Crystallographic analysis of virions shows the same surface features as observed in the empty capsid but does not reveal details of the nucleohistone complex structure, since the analysis imposes icosahedral symmetry on the entire virus particle and the core may not be organized with this symmetry.



The tubular forms of Py (and Shope papilloma and human wart) have been studied by electron microscopy and image analysis. Two general classes of tubes are observed: narrow (≈30 nm diameter) built of pentamers and wide (≈45–50 nm) originally thought to contain hexameric morphological units. A reexamination of the wide tubes using minimal-irradiation electron microscopy and image analysis (Baker et al., 1983; and Fig. 20.6) revealed that all tubes are assemblies of paired capsomers with a simple relationship between the packing arrangements of pentamers in the different tubes.

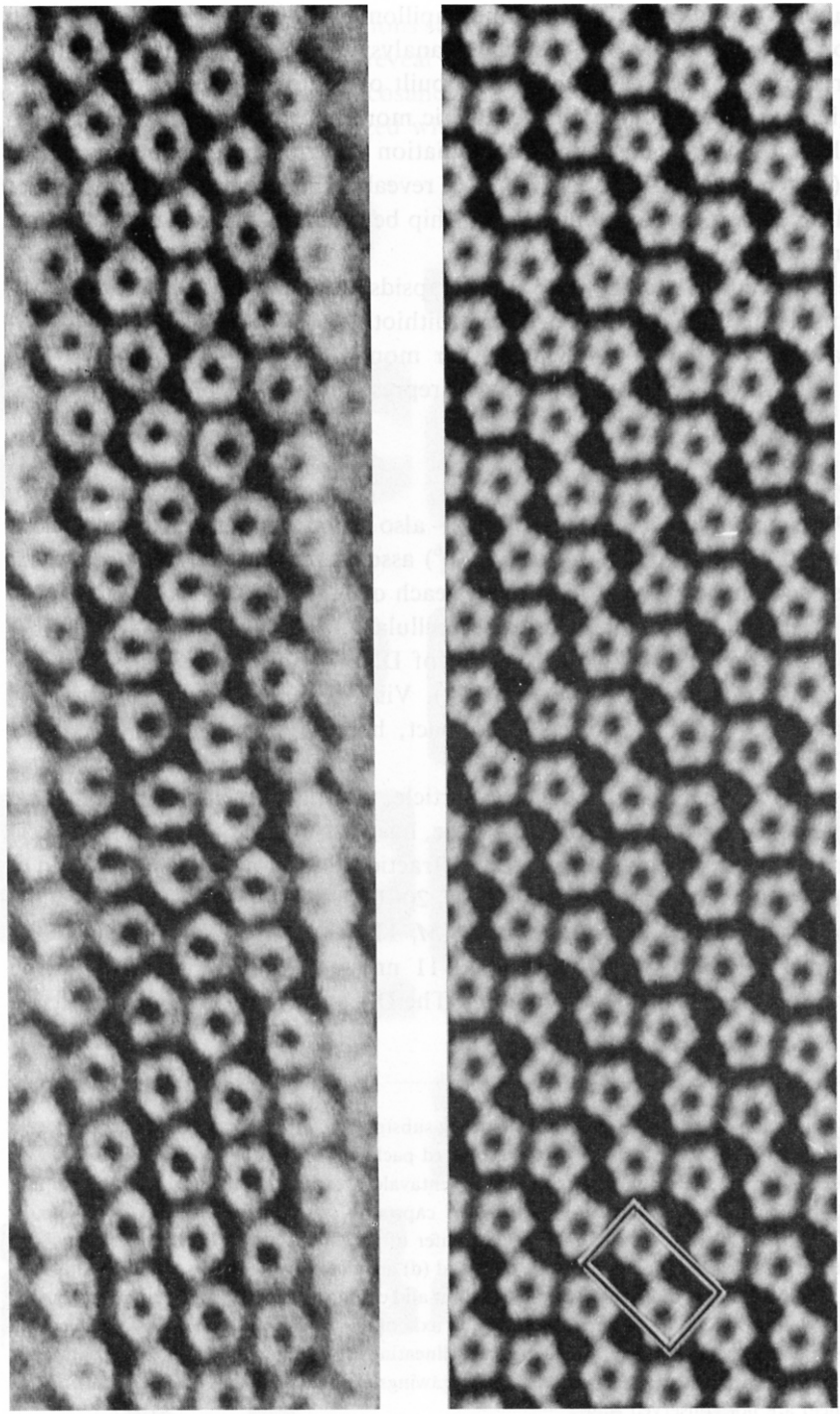
Gentle dissociation of the virions and capsids with EGTA ([ethylenebis(oxyethylenenitrile)]tetraacetic acid) or DTT (dithiothreitol) produces isolated morphological units. There is no evidence for monomers or hexamers of VP1 in solution, implying that the pentamer may represent a stable functional form of VP1.

Nucleoprotein complex

The DNA/protein complex in Py and SV40 – also called a minichromosome – contains a duplex DNA molecule (M_r 3.5×10^6) associated with about 25 octameric aggregates of cellular histones (two copies each of H3, H4, H2A and H2B). The primary repeating unit of both viral and cellular chromatin is the nucleosome, which contains a fairly well-defined length of DNA (≈200 basepairs) associated with the histone octamer (Kornberg, 1977). Viral chromatin lacks histone H1, which presumably helps form more compact, higher-ordered states of cellular chromatin (Thoma et al., 1979).

A well-characterized nucleosome core particle, produced by limited digestion of cellular chromatin with micrococcal nuclease, has been studied extensively by electron microscopy and X-ray and neutron diffraction techniques (e.g. Richmond et al., 1984). The nucleosome core particle (M_r 206 000) contains 146 (±2) basepairs of duplex DNA and the histone octamer (M_r 110 000), and is a flat, somewhat wedge-shaped cylinder (5.7 nm high and 11 nm in diameter) with a bipartite character and a two-fold axis of symmetry. The DNA is wound around the outside

Fig. 20.5. Sections of the electron-density map showing substructure of the capsomers (a–d) and drawings on a polyhedral surface (e,f) illustrating an inferred packing relationship of structure units. Left column: views down the five-fold axis through a pentavalent capsomer (same orientation as Fig. 20.4a,b). Right column: views through a hexavalent capsomer (same orientation as Fig. 20.4c,d). Panels (a) and (b) are sections 21.5 nm above the center of the capsid at a level where there is little contact between neighboring capsomers. Panels (c) and (d) are 19.5 nm above the center. At this level and below, the five subunits of each capsomer splay out and contact the basal parts of the neighboring subunits. The section planes are perpendicular to the axis of the central capsomer and slice through the neighboring capsomers obliquely. The polygons delineating the domain of the central capsomer are marked by dotted lines in a–d. In (e) and (f), the drawings illustrate that the interactions between subunits (mice) are not conserved.



a b

of the histone octamer in 1.8 turns of a flat superhelix with a pitch of 2.8 nm. The histone octamer, either associated with DNA in nucleosome core particles (Richmond et al., 1984) or in tubular aggregates free of DNA (Klug et al., 1980), is wedge-shaped with a roughly circular outline ($\approx$7 nm diameter) at its broadest aspect and, at right angles to this, is $\approx$5.6 nm at its thickest point.

The specific organization of the minichromosome inside the virus is not known, although it must be in a condensed state, since it is confined to an approximately spherical space of diameter 30–32 nm defined by the capsid. It is not known whether the viral chromatin adopts a higher-order structure such as the solenoid of cellular chromatin (Finch and Klug, 1976), nor is it known what specific interactions between the nucleohistone and viral proteins (VP1, VP2 and VP3) are important for assembling stable, infectious particles. A hypothetical model for the structure of the nucleohistone core of SV40 virus was proposed (Martin, 1977) in which 21 nucleosomes could be packed, with little or no perturbation of their structure, inside the capsid. In the model, a single nucleosome at the center of the virus is surrounded by 20 others, each beneath one of the 20 faces of the icosahedral protein shell.

20.4. Antigenic properties

Py and SV40 each occur in a single antigenic type immunologically unrelated to each other. There is a certain level of cross-reactivity between Py and SV40 VP1 (slightly less with BKV and JCV). T (tumor) antigens (see following section) do not cross-react with capsid protein. The T antigens of BKV, JCV and SV40 unexpectedly all cross-react, with the cross-reaction generally much stronger than that detected between their V antigens (Tooze, 1981).

20.5. Biological properties

Lytic infection of permissive cells

The infection cycle of Py is initiated by adsorption and pinocytosis of virions into permissive cells such as mouse embryo or kidney. Virions lose capsid protein and the DNA may associate with host proteins and enter the nucleus. The limited DNA coding potential means that the cell supplies the enzymes and factors required for

Fig. 20.6. (a) Computer-filtered image of the front layer of a flattened wide tube similar to the tube at the left in Fig. 20.1A. (b) Computed model of a single layer of regular pentamers arranged with the same lattice parameters observed in (a). One choice of unit cell is boxed.

viral replication, transcription and translation. Infection alters normal cellular functions: resting cells reenter the cell cycle and the entire complement of host DNA is replicated and cellular protein synthesis is enhanced.

Early expression of the viral genome leads to production of non-structural viral proteins (T antigens). The large T antigen is required for induction of host-cell functions and initiation of viral DNA replication; the expression of late genes results in the appearance primarily of the structural proteins (VP1, VP2, VP3). All of the viral mRNAs are products of splicing. The VP1 amino-terminal region is encoded by the same DNA that codes for the carboxyl ends of VP2 and VP3. The different amino acid sequences between VP1 and VP2/VP3 in these overlapping regions result from translation of the mRNAs in different reading frames. The coding regions of VP2 and VP3 overlap and are translated in the same reading frame; VP3 is identical to the carboxy-terminal half of VP2. The three Py T antigens all share identical amino-terminal sequences.

The relationship between the early and late phases of viral replication are still unclear. However, it appears that the middle T antigen which functions as an ATPase is indirectly necessary to enhance the phosphorylation of VP1. This modification is required for assembly, since mutants which are defective in the large T antigen still synthesize appropriate levels of the viral DNA and viral coat proteins but do not produce normal levels of mature virion (Garcea and Benjamin, 1983).

The pathway for assembly of mature virions has been studied in detail for SV40 and is similar for Py. Viral proteins are synthesized in the cytoplasm and then transported through the nuclear membrane into the nucleus, where assembly occurs. A 75 S nucleoprotein complex, consisting of a single molecule of the closed circular viral DNA with cellular histones and a small amount of capsid proteins, is the first assembly intermediate observed. VP1 and VP3 are added to this nucleoprotein complex to form a 200 S previrion intermediate. Further addition of VP1, VP2 and VP3 leads to a 250 S intermediate which is unstable in high salt. The final steps involved in the maturation of the 250 S particle are unknown but may include the formation of intersubunit disulfide cross-links.

Virions remain in the nucleus or move to the cytoplasm after rupture of the nuclear membrane and accumulate in cytoplasmic vacuoles awaiting cell lysis for release.

Non-lytic infection and transformation of non-permissive cells

Py is widespread in mouse populations both in the wild and in laboratories. The virus is normally transmitted through animal excretions or secretions. It has a broader range of oncogenicity than SV40, transforming not only mouse cells but also cultured cells of rat, rabbit, guinea pig, dog, cow, monkey and man. Innoculation into animals results, after a latent period of several months, in tumors in virtually all organs and tissues except the brain.

Viral DNA integrates into cellular DNA, generally at several sites (≈1–10 genome equivalents/diploid quantity of cellular DNA), with no specificity for the site of integration and no preferred site at which the circular viral genome is opened to promote integration. The entire viral genome is not required for integration or transformation. Cells often contain both integrated and non-integrated viral genomes. Integration does not appear to be a requirement for transformation: expression of the viral genome is.

20.6. Other Papovaviruses

Py and SV40 are morphologically indistinguishable (Fig. 20.1) and share marked similarities in most other physical, chemical, genetic and biological properties. Nevertheless, there are distinct differences between the two viruses. The DNA sequences differ except for remarkable conservation at the viral origin of DNA replication (including BKV). SV40, BKV and JCV lack the middle T antigen. SV40 grows well only in primary cells of African green monkey kidneys, certain other monkey cells and cell lines derived therefrom. It does not grow well in its persistently infected host (rhesus monkey kidney cells). The human viruses, BK and JC, have the most limited host range of the polyomaviruses. JC was the first human virus which could be shown to induce a tumor in a primate.

Papillomaviruses cause benign skin tumors (warts) in a variety of mammalian species which serve as natural hosts. In rabbits the tumors regularly become malignant if they persist for a sufficiently long time; occasionally separate warts regress spontaneously, suggesting an immunological mechanism. The structure of papillomaviruses has been extensively studied by electron microscopy. At the limits of resolution of the technique, these viruses appear to have a morphology similar to that of the smaller polyomaviruses. Recent evidence suggests that SV40 has a 72-pentamer capsid structure similar to Py (Baker et al., 1985). Thus the capsid structure of all papovaviruses, including other members of the polyoma and papilloma genera, may be a highly conserved phenotype.

Acknowledgements

This chapter is dedicated to D.L.D. Caspar and W.T. Murakami for years of encouragement and support. We thank M. Bina for valuable suggestions, P. Flicker and P.N.T. Unwin for expert assistance in the photography of frozen-hydrated SV40 samples, W. Boyle, J. Black and N. Olson for photography and M. Radermacher for programs. This manuscript was funded in part by NIH grant GM33050 to T.S.B. Fig. 20.2B was imaged with the Brookhaven STEM with funds from NIH Biotechnology Resource Grant RR 01777.

Suggested reading

Baker, T.S., Caspar, D.L.D. and Murakami, W.T. (1983) Polyoma virus 'hexamer' tubes consist of paired pentamers. Nature 303, 446–448.

Baker, T.S., Drak, J. and Bina, M. (1985) Cryo-electron microscopy and image analysis of SV40. Proc. 43rd Annu. Meet. Electron Microsc. Soc. Am. 43, 316–317.

Caspar, D.L.D. and Klug, A. (1962) Physical principles in the construction of regular viruses. Cold Spring Harb. Symp. Quant. Biol. 27, 1–24.

Finch, J.T. and Klug, A. (1976) Solenoidal model for superstructure in chromatin. Proc. Natl. Acad. Sci. USA 73, 1897–1901.

Finch, J.T. and Crawford, L.V. (1975) Structure of small DNA-containing animal viruses. In Comprehensive Virology, Eds. H. Fraenkel-Conrat and R.R. Wagner; Vol. 5, pp. 119–154. Plenum Press, New York.

Garcea, R.C. and Benjamin, T.C. (1983) Host range transforming gene of polyoma virus plays a role in virus assembly. Proc. Natl. Acad. Sci. USA 80, 3613–3617.

Horne, R.W. and Pasquali-Ronchetti, I. (1974) A negative staining–carbon film technique for studying viruses in the electron microscope. I. Preparative procedures for examining icosahedral and filamentous viruses. J. Ultrastruct. Res. 47, 361–383.

Klug, A., Rhodes, D., Smith, J., Finch, J.T. and Thomas, J.O. (1980) A low resolution structure for the histone core of the nucleosome. Nature 287, 509–516.

Kornberg, A. (1977) Structure of chromatin. Annu. Rev. Biochem. 46, 931–954.

Lancaster, W.D. and Olson, C. (1982) Animal papillomaviruses. Microbiol. Rev. 46, 191–207.

Martin, R.G. (1977) On the nucleoprotein core of simian virus 40. Virology 83, 433–437.

Perbal, B.V., Linke, H.K. and Fareed, G.C. (1983) Polyomaviruses. In Molecular Biology of Polyomaviruses and Herpesviruses, pp. 1–59. Wiley-Interscience, New York.

Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D. and Klug, A. (1984) Structure of the nucleosome core particle at 7 Å resolution. Nature 311, 532–537.

Rayment, I. (1984) Animal Virus Structure. In Biological Macromolecules and Assemblies, Eds. F.A. Jurnak and A. McPherson; Virus Structures, Vol. 1, pp. 255–298. Wiley-Interscience, New York.

Rayment, I., Baker, T.S., Caspar, D.L.D. and Murakami, W.T. (1982) Polyoma virus capsid structure at 22.5 Å resolution. Nature 295, 110–115.

Thoma, F., Koller, Th. and Klug, A. (1979) Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. J. Cell Biol. 83, 403–427.

Tooze, J. (1981) DNA Tumor Viruses, Molecular Biology of Tumor Viruses, 2nd Edn., Part 2/Revised. Cold Spring Harbor Laboratory, NY.