1 Towards a definition of a virus

Fig. 1.1 Plaques of viruses. (a) Plaques of a bacteriophage on a lawn of *Escherichia coli*. (b) Local lesions on a leaf of *Nicotiana* caused by tobacco mosaic virus. (c) Plaques of influenza virus on a monolayer culture of chick embryo fibroblast cells.

Fig. 1.2 A one-step growth curve of bacteriophage λ following infection of susceptible bacteria (*Escherichia coli*). During the *eclipse phase* (1), the infectivity of the cell-associated, infecting virus is lost as it uncoats; during the *maturation phase* (2) infectious virus is assembled inside cells (cell-associated virus), but not yet released; and the *latent phase* (3) measures the period before infectious virus is released from cells into the medium. Total virus is the sum of cell-associated virus + released virus. Cell-associated virus decreases as cells are lysed. This classic experiment shows that phages develop intracellularly.

Fig. 1.3 A diagrammatic representation of the six phases common to all virus multiplication cycles. See text for details.

Fig. 1.4 The Hershey–Chase experiment proving that DNA (labelled with ^{32}P) is the genetic material of bacteriophage T2.

Fig. 1.5 The experiment of Fraenkel-Conrat and Singer which proved that RNA is the genetic material of tobacco mosaic virus.

Fig. 2.1 Cytopathic effects caused by an influenza A virus and human respiratory syncytial virus (HRSV) in confluent cell monolayers (a layer of cells with a depth of just one cell). (a) Chick embryo cells infected by influenza A virus. In the clear central area infected cells have lysed. Some cell debris remains, and cells in the process of rounding up can be seen on the edge of the lesion. There are healthy cells around the periphery. (b) A monkey cell line infected with HRSV. HRSV does not lyse cells, but causes them to fuse together to form syncytia. A collection of syncytia forms the dark area in the center. Individual cells are magnified to approximately 3 mm in length, and are packed close together. Note the difference in morphology between the monkey cells and the chick cells: the monkey cells are slimmer and are more regularly packed together.

Fig. 2.2 Preparation of tracheal organ cultures.

Fig. 2.3 Sections through tracheal organ cultures: (a) uninfected; (b) infected with a rhinovirus for 36 hours. Note the disorganization of the ciliated cells (uppermost layer) after infection. (Courtesy of Bertil Hoorn.)

Fig. 2.4 Cell culture.

Fig. 2.5 (a) One way to increase cell density is by increasing the surface area to which cells can attach; this is a view from the end of a bottle lined with spiral plastic coils. The bottle is rotated slowly, at about 5 rev/h, so that a small volume of culture fluid can be used. Cells tolerate being out of the culture fluid for short periods. (b,c) Cells growing on microcarrier beads. (b) Scanning electron micrograph of pig kidney cells. (Courtesy of G. Charlier.) (c) Removal of cells from a microcarrier bead by incubation with trypsin. The cells are rounding up and many have already detached. Each bead is about 200 μm in diameter. The microcarriers shown are Cytodex (Pharmacia Ltd) (reproduced by permission).

Fig. 2.6 A neutralization test. Virus A loses its infectivity after combining with A-specific antibody (it is neutralized). A-specific antibody does not bind to virus B, so infectivity of virus B is unaffected. The complete test requires the reciprocal reactions.

Fig. 2.7 Hemagglutination titration. Here an influenza virus is serially diluted from left to right in wells in a plastic plate. Red blood cells (RBCs) are then added to 0.5% v/v and mixed with each dilution of virus. Where there is little or no virus, RBCs settle to a button (from 1/128) indistinguishable from RBCs to which no virus was added (row 3). Where sufficient virus is present (up to 1/64), cells agglutinate and settle in a diffuse pattern. (Photograph by Andy Carver.)

Fig. 2.8 In the hemagglutination–inhibition test, antibody is diluted from left to right. Four hemagglutination units (HAUs) of an influenza virus are added to each well. The antibody–virus reaction goes to completion in 1 hour at 20°C. Red blood cells are then added to detect virus that has not bound antibody. In this test, hemagglutination is inhibited up to an antibody dilution of 1/3200. (Photograph by Andy Carver.)

Fig. 2.9 Fluorescent antibody staining. An antibody covalently bound to a fluorescent dye has been used to detect an antigen present mainly in the nucleus (arrows) of influenza virus-infected cells.

Fig. 2.10 Identification of an unknown virus by ELISA with a specific antiserum. The unknown virus would be used in parallel with a control virus preparation that is known to react with the antibody. The unknown virus is positively identified when the antibody reaction is identical to that using the control virus.

Fig. 2.11 An outline of the polymerase chain reaction (PCR). Step 1: denaturation; step 2: annealing of oligonucleotide primers; step 3: synthesis of new DNA by added polymerase. This is repeated *n* times. Note that end-product, the amplified DNA fragment or amplicon, is not formed until after the third annealing process.

3 The structure of virus particles

Fig. 3.1 Electron micrographs of virus particles showing their regular shape: rods and spheres. (a) Tobacco mosaic virus. (b) Bacteriophage Si1.

Fig. 3.2 Arrangement of identical asymmetrical components around the circumference of a circle to yield a symmetrical arrangement.

Fig. 3.3 Symmetrical arrangement of identical asymmetrical subunits by placing them on the faces of objects with cubic symmetry. (a) Asymmetrical subunits located at vertices of each triangular facet. (b) Asymmetrical subunits placed at vertices of each square facet. (c) Arrangement of asymmetrical subunits placed at each corner of a cube with faces as represented in (b).

Fig. 3.4 (a) Properties of a regular icosahedron. Each triangular face is equilateral and has the same orientation whichever way it is inserted. Axes of symmetry intersect in the middle of the icosahedron. There are 12 vertices, which have fivefold symmetry, meaning that rotation of the icosahedron by one-fifth of a revolution achieves a position such that it is indistinguishable from its starting orientation; each of the 20 faces has a threefold axis of symmetry and each of the 30 edges has a twofold axis of symmetry (b). The icosahedron is built up of five triangles at the top, five at the bottom, and a strip of 10 around the middle (c). (Copyright 1991, from *Introduction to Protein Structure* by C. Branden & J. Tooze. Reproduced by permission of Routledge Inc., part of The Taylor & Francis Group.)

Fig 3.5 (a) Spatial arrangement of two identical sets of subunits. Note that any member of the set represented by dark spheres does not have the same neighbors as a member of the other set represented by light spheres. (b) An example of a geodesic dome – the United States Pavilion at Expo '67 in Montreal. (Courtesy of the US Information Service.)

Fig. 3.6 Arrangement of 60n identical subunits on the surface of an icosahedron. (a) $n = 1$ and the 60 subunits are distributed such that there is one subunit at the vertices of each of the 20 triangular faces. Note that each subunit has the same arrangement of neighbors and so all the subunits are equivalently related. (b) $n = 4$. Each triangular face is divided into four smaller, but identical, equilateral triangular facets and a subunit is again located at each vertex. In total, there are 240 subunits. Note that, in contrast to the arrangements discussed in Box 3.1, each subunit, whether represented by a dark or light sphere, has the identical arrangement of neighbors: see the face in which triangles 1–4 have been drawn. However, since some subunits are arranged in pentamers and others in hexamers, the members of each set are "quasi-equivalently" related.

Fig. 3.7 Schematic diagram of a $T = 3$, 180-subunit virus. Each triangle is composed of three subunits, A, B, and C, which are asymmetrical by virtue of their relationship to other subunits (pentamers or hexamers). (Copyright 1991, from *Introduction to Protein Structure* by C. Branden & J. Tooze. Reproduced by permission of Routledge Inc., part of The Taylor & Francis Group.)

Fig. 3.8 (a) The linear arrangement of domains present in the single virion polypeptide of tomato bushy stunt virus. (b) Conformation of the polypeptide. The S domain forms the shell of the virion, while P points outwards and R is internal. (c) This shows a triangular face, composed of subunits A, B, and C, and the interaction of the P domains to form dimeric projections (see text). (Copyright 1991, from *Introduction to Protein Structure* by C. Branden & J. Tooze. Reproduced by permission of Routledge Inc., part of The Taylor & Francis Group.)

Fig. 3.9 The jelly-roll or antiparallel β barrel: a common structure of plant and animal virion proteins. The formation of an antiparallel β barrel from a linear polypeptide can be visualized to occur in three stages. First, a hairpin structure forms where β strands are hydrogen-bonded to each other: 1 with 8, 2 with 7, 3 with 6, and 4 with 5, creating antiparallel β strand pairs separated by loop regions of variable length (a). Second, these pairs become arranged side by side, so that further hydrogen bonds can be formed by newly adjacent strands, e.g. 7 with 4 (b) Third, the strand pairs wrap around an imaginary barrel forming a three-dimensional structure. The eight β strands are arranged in two sheets, each composed of four strands: strands 1, 8, 3, and 6 form one sheet and strands 2, 7, 4, and 5 form the second sheet (c). The dimensions of the barrel are such that each protein forms a wedge, and these wedges are subunits that are assembled into virus particles (d). ((a–c) Copyright 1991, from *Introduction to Protein Structure* by C. Branden & J. Tooze; reproduced by permission of Routledge Inc., part of The Taylor & Francis Group. (d) From Hogle *et al.*, 1985 *Science* **229**, 1358.)

Fig. 3.10 A picornavirus particle (a) and the attachment site formed by VP1 shown as an annulus around the fivefold axis of symmetry. (b) A vertical section through a VP1 pentamer where the cross-section of the annulus is referred to as the "canyon." ((a) From Smith *et al.*, 1993 *Journal of Virology* **67**, 1148. (b) From Luo *et al.*, 1987 *Science* **235**, 182.)

Fig. 3.11 The structure of adenoviruses. (a) A negatively stained electron micrograph of an adenovirus. (b) Model of an adenovirus to show arrangements of the capsomers. (c) Schematic diagram to show the arrangements of the subunits on one face of the icosahedron. Note the subdivision of the face into 25 smaller equilateral triangles. (Photographs courtesy of Nicholas Wrigley.)

Fig. 3.12 The double capsid structure of reovirus showing the location of polypeptides in the virion. Here a section of the virion has been taken through the fivefold axis of symmetry of the vertex. In order to demonstrate protein function, two intermediates that occur during the virus uncoating process are also shown: the intermediate subviral particle (ISVP) and the viral core. In the ISVP, σ1 has achieved an extended conformation and σ3 has been lost from the outer capsid. Some molecules of σ1 may also be extended in the virion, but this is seen more frequently in the intermediate. The core is formed by the loss of the entire outer capsid and the change in conformation of the turret protein λ2 to form the channel through which mRNA synthesized in the particle will escape into the cell cytoplasm. (From M. L. Nibert *et al.* in B. N. Fields, D. M. Knipe and P. M. Howley eds., 1996 *Virology*, Lippincourt-Raven, p. 1562.)

Fig. 3.13 Sindbis virus: an enveloped icosahedron. The core is $T = 3$, but the envelope is $T = 4$. See text for the explanation. (Courtesy of S. D. Fuller.)

Fig. 3.14 Influenza A virus (an orthomyxovirus) and vesicular stomatitis virus (a rhabdovirus): viruses with enveloped helical structures. Although their morphology is different, these viruses are constructed in the same way. (a) Negatively stained electron micrograph of influenza A virus showing the internal helical ribonucleoprotein (RNP) core and the surface spikes. (b) Aggregates of purified neuraminidase. (c) Aggregates of purified hemagglutinin. Note the triangular shape of the spikes when viewed end-on. (d) Schematic representation of the structure of influenza virus. (e) Negatively stained electron micrograph of vesicular stomatitis virus. (f) Schematic representation of the structure of vesicular stomatitis virus. (Electron micrographs courtesy of Nicholas Wrigley and Chris Smale.)

Fig. 3.15 The influenza virus hemagglutinin (HA). This is a homotrimer but only a monomer is shown here. The HA is synthesized as a single polypeptide which is proteolytically cleaved into the membrane-bound HA2 and the distal HA1. (a) An outline structure showing that HA1 and HA2 are both hairpin structures. (b) The crystal structure. The globular head of HA1 bears all the neutralization sites (A–E; shaded) and is made of a distorted jelly-roll β barrel like most of the icosahedral viruses. (From Wiley *et al.* (1981) *Nature (London)*, **289**, 373.)

Fig. 3.16 Electron micrograph of bacteriophage T2 with six long tail fibers. Tail pins are not evident but a short fiber (indicated by the arrow) can be seen. The bar is 100 nm. (Courtesy of L. Simon.)

Fig. 4.1 The Baltimore classification scheme (see text for details). mRNA is designated as positive sense. Translation of protein from mRNA and positive sense RNA virus genomes is indicated with red arrows. The path of production of mRNA from double-stranded templates is shown with black arrows. Green arrows show the steps in the replication of the various types of genome with double-headed arrows, indicating production of a double-stranded intermediate from which single-stranded genomes are produced.

Fig. 4.2 Diagram of the circular single-stranded 359-nucleotide RNA of potato spindle tuber viroid, showing the maximized base-paired structure.

5 The process of infection: I. Attachment of viruses and the entry of their genomes into the target cell

Fig. 5.1 (a) "Lipid sea" model of plasma membrane structure. (Adapted from Singer & Nicholson 1972 *Science* **175**, 723.) Proteins (P) may span the lipid bilayer or may not, and are free to move laterally like icebergs in the sea. (b) Pinocytosis by a plasma membrane (pm) inwards (A,B,C) and (c) exocytosis outwards (D,E,F).

Fig. 5.2 Entry of animal virus genomes into cells. All viruses start by attaching to specific receptors on cells. (a) Entry by fusion of the lipid bilayers of an enveloped virus and the plasma membrane at neutral pH. (b) Entry by endocytosis is followed by a fusion of the vesicle with an endosome and a decrease in the pH of the endosome. This promotes conformational changes in viral proteins. For enveloped viruses (upper panel) this leads to fusion of the lipid bilayers of the virus and the endosome. For nucleocapsid virus particles (lower panel), the low pH causes conformational changes in viral proteins. This results in the insertion of newly exposed hydrophobic regions of the virion into the lipid bilayer of the vesicle and the escape of the viral genome and associated proteins into the cytoplasm.

Fig. 5.3 Model for the fusion entry of the genome of an HIV-1 virion into a target cell. The long C-terminal tail of gp41 is not shown. (Adapted from Moore J. P. and Doms R. W. (2003) *Proceedings of the National Academy of Sciences of the USA* **100**, 10598.)

Fig. 5.4 A model for the translocation of poliovirus RNA across the cell membrane. (Adapted from Belnap D. M. *et al*. (2000) *Journal of Virology* **74**, 1342.)

Fig. 5.5 Correlation of phage sensitivity with lipopolysaccharide structure of the cell wall. The upper part of the diagram shows the structure of the lipopolysaccharide in different mutants of *Salmonella typhimurium*. Abbreviations: glc, glucose; gal, galactose; GNac, *N*-acetylglucosamine; hep, heptose; KDO, 2-keto-3-deoxyoctonic acid. The lower panel shows the sensitivity of the different mutants to several bacteriophages. Abbreviations: S, sensitive; R, resistant.

Fig. 5.6 Steps in the attachment of bacteriophage T4 to the cell wall of *Escherichia coli*. (a) Unattached phage showing tail fibers and tail pins (compare Fig. 3.16). (b) Attachment of the long tail fibers. (c) The phage particle has moved closer to the cell wall and the tail pins are in contact with the wall.

Fig. 5.7 Attachment of a bacteriophage χ to the filament of a bacteria flagellum. (Courtesy of J. Adler.)

Fig. 5.8 Attachment of many spherical RNA phages to the sex pilus of *Escherichia coli.* (Courtesy of C. C. Brinton.)

Fig. 5.9 Representation of the mechanism of entry of the phage T4 genome into the bacterial cell. (a) The phage tail pins are in contact with the cell wall and the sheath is extended. (b) The tail sheath has contracted and the phage core has penetrated the cell wall; phage lysozyme has digested away the cell beneath the phage. (c) Negatively stained electron micrograph of T4 attached to an *Escherichia coli* cell wall, as seen in thin section. The needle of one of the phages just penetrates through the cell wall (arrow). Thin fibrils extending on the inner side of the cell wall from the distal tips of the needles are probably DNA.

6 The process of infection: IIA. The replication of viral DNA

Fig. 6.1 Model for replication of double-stranded DNA through discontinuous synthesis of the lagging strand. Both strands are synthesized in a 5′ → 3′ direction but only the leading strand is synthesized continuously, the other being formed from a series of short DNA molecules, each primed by a piece of RNA. The primers are excised and replaced by DNA before the fragments are joined by DNA ligase. Green, dark blue: parental DNA; blue: new DNA; pink: RNA primer.

Fig. 6.2 The problem of replicating the ends of linear DNA molecules through the use of RNA primers. Once the first primer has been excised, there is no mechanism for filling the gap. Green: parental DNA; blue: new DNA; pink: RNA primer.

Fig. 6.3 (a–f) A general scheme for SV40 replication (see text for details). Green: parental DNA; blue: new DNA; pink: RNA primer. Arrowheads on newly synthesized DNA fragments represent 3′ ends; the 5′ → 3′ polarity of other DNA strands is indicated by > symbols.

Fig. 6.4 (a) HSV1 genome organization: L, long; S, short; U, unique sequence; TR, terminally repeated sequence; IR, internally repeated sequence. Black triangles represent direct repeats (A) at the genome termini and their internal inverted copies (A′). Red and blue shading represent the remainder of the inverted repeat sequences that flank the long and short unique regions respectively. The genome is not drawn to scale; U_S should represent about 8.5% and U_L about 71% of the total genome. (b,c) The HSV1 genome is depicted by two parallel lines, with terminal direct repeats shown as **A** and its complement **A′**, the repeats flanking the long unique region as **EF** and its complement **E′F′**, and the repeats flanking the short unique region as **XY** and its complement **X′Y′**. 3′ ends are represented by arrowheads. (b) Experimental demonstration of direct repeats at the HSV1 genome termini (Box 6.4). (c) Experimental detection of inverted repeats in HSV1 DNA (Box 6.4). The two forms of self-annealed strand that are seen are shown for the upper strand of the double-stranded genome. The same forms may be adopted by the lower strand.

Fig. 6.5 A model for the replication of HSV1 DNA (see Section 6.3 for details). Green: parental DNA; blue: new DNA; pink: RNA primer; purple: assembling capsid.

Fig. 6.6 Demonstration of inverted terminal repeat sequences in adenovirus DNA. The genome is depicted by two parallel lines, with inverted terminal repeats shown as JK and its complement J′K′. 3′ ends are represented by arrowheads. Denaturation and annealing of the genome allows formation of single-stranded circles with double-stranded "pan-handle" projections.

Fig. 6.7 (a–i) A general scheme for adenovirus DNA replication (see Section 6.4 for details). Green: parental DNA; blue: new DNA. Arrowheads on new DNA strands represent 3′ ends. Red circles represent the terminal protein (TP) attached to parental DNA 5′ ends and purple circles the terminal protein precursor molecules (pTP) which prime new DNA synthesis. The viral DNA polymerase is represented in pink.

Fig. 6.8 (a–f) General scheme for vaccinia virus DNA replication (see Section 6.4 for details). Green: parental DNA; blue: new DNA. Arrowheads on new DNA strands represent 3′ ends. Complementary sequences are denoted **A**, **A′** etc. Note that the two alternative forms of the terminal loop sequence, **B**, **B′** are exchanged with each of replication; individual molecules may have loops with identical or complementary sequences.

Fig. 6.9 (a,b) Schematic representation of the genome structures of autonomous and defective parvoviruses. Complementary sequences are denoted A, A′ etc.

Fig. 6.10 (a–h) A scheme for the replication of an autonomous parvovirus (see Section 6.6 for details). Green: parental DNA; blue: new DNA; purple: assembling capsid. Arrowheads represent 3′ ends. The orange circle represents the viral site-specific nicking enzyme. Complementary sequences are denoted A, A′ etc.

Fig. 7.1 Hypothetical schemes to explain the generation of DI RNAs having sequences identical with both the 5′ and 3′ regions of the genome (a) and with the 5′ region only (b).

Fig. 7.2 Comparison of the genome organizations of normal and DI virus. (a) A representation of a normal virus genome. The colored segments, labelled A to D, represent different regions of the genome (not to scale). (b) The genome of a 5′, 3′ DI virus in which a large portion of the genome (region C) has been deleted. (c) The genome of a 5′ DI virus. Large portions of the genome (regions C and D) are not present and the stippled red block (region A′) represents the complement of the normal 5′ end sequence of region A. (d) A complex DI virus genome containing multiple copies of a short portion (region B) of the normal genome with other sequences (region C) deleted.

Fig. 7.3 Diagrammatic representation of the experiment that demonstrates that the reovirus genome is replicated conservatively. Radioactively labeled RNA strands are represented by red lines and unlabeled strands by black lines. See Box 7.2 for details.

Fig. 7.4 Circularization of picornavirus genome RNA in the replication complex.

Fig. 7.5 Replication of picornavirus genome RNA. Note that the replication takes place using a template which is held in a circular form by the action of three proteins but is represented here as linear for simplicity. (a) Proposed structure for a molecule of replicative intermediate before deproteinization; (b) after deproteinization to generate a replicative intermediate (RI); (c) the effect of treating deproteinized RI with RNase to produce a replicative form (RF) RNA.

8 The process of infection: IIC. The replication of RNA viruses with a DNA intermediate and vice versa

Fig. 8.1 Retroviral heterodimeric reverse transcriptase. The enzyme from human immunodeficiency virus is formed of p66 and p51 subunits. P66 has five structural domains, colored blue, red, green, yellow, and orange in order from the N-terminus. The last of these is the RNaseH domain, not present in p51. The remaining four domains found in p51 are colored in pale versions of the equivalent p66 domains. The structural arrangement of these domains in p51 is distinct from their organization in p66, where they form the active site for DNA synthesis. (Drawn from data presented in Ding C. H. *et al*. (1998). *Journal of Molecular Biology* **284**, 1095–1114.)

Fig. 8.2 Comparison of the structures of retrovirus genome RNA (top) and the proviral DNA created from it by reverse transcription. U5 and U3 are unique sequences at the 5′ and 3′ ends of virion RNA; R is a directly repeated sequence at the RNA termini. Short inverted repeat sequences are represented as ◀, ▶. tb is the binding site for a transfer RNA and polyP is a polypurine region, both significant in reverse transcription (Section 8.4 and Fig. 8.3). Long terminal repeats (LTRs) comprise duplications of the sequences U3, R, U5. psi is the specific packaging signal for RNA genomes. Not to scale.

Fig. 8.3 (a–j) A scheme for the synthesis of retroviral linear proviral DNA by reverse transcriptase (Section 8.4 for details). Orange lines: RNA; yellow lines: (−)DNA; red lines: (+)DNA; green ellipses: reverse transcriptase enzyme. Arrowheads represent 3′ ends. The color coding of the U3, R, and U5 elements of the LTRs is carried forward from Fig. 8.2 to illustrate how the LTRs are created. Abbreviations and other symbols as for Fig. 8.2.

Fig. 8.4 (a–e) Integration of retroviral DNA into the host genome. For details see Section 8.5. Mid, dark, and light blue: the U3, R, and U5 elements of the retroviral LTRs; dark green: retroviral DNA; yellow: host DNA random integration target; dark pink: DNA repair synthesis.

Fig. 8.5 (a–h) Replication of the hepadnavirus genome by reverse transcription (see Section 8.9 for details). Orange: RNA; blue: (+)DNA; green: (−)DNA; purple sphere: RNA cap structure; AAA: RNA polyA tail. DR1, DR2 represent two copies of a short directly repeated sequence in the genome. The viral P protein (terminal protein and reverse transcriptase) attached to the genome 5′ end is shown as a blue sphere. Filled arrowheads represent 3′ ends. Other arrows indicate the polarity (5′ → 3′) of nucleic acid strands.

Fig. 9.1 Map of the genome of SV40 virus showing the positions of the early and late transcription units. P denotes promoters of transcription and ori denotes the origin of replication. The mRNAs are shown in detail in Figs 9.2 and 9.3, and the control region in Fig. 9.4.

Fig. 9.2 Early gene expression by (a) SV40 and (b) polyoma virus. Differential splicing of the primary transcript in each case produces mRNAs with distinct coding capacities (see text and Box 9.2 for details). Red lines denote DNA and blue lines, RNA; dotted lines denote introns and thin lines untranslated RNA regions. Proteins are represented in linear form, with color coding to indicate the sequences that are common between two or more proteins and numbers indicating amino acid residues.

Fig. 9.3 Late gene expression by SV40 virus. The late mRNAs have heterogeneous 5′ ends because the late promoter lacks a TATA box, which normally serves to direct initiation to a specific location. Legend details as for Fig. 9.2. Polyoma virus is very similar but the virion protein (VP1) coding region does not overlap with that of VP2/VP3.

Fig. 9.4 The control region from laboratory strains of SV40. A number of sequence features are evident in the DNA and these correspond to functional units as indicated. Sites 1, 2, and 3 are three DNA regions, each containing multiple binding sites for large T antigen.

Fig. 9.5 The genome organization of bovine papillomavirus type 1. E1–E8, L1, and L2 represent open reading frames. Reading frames were originally designated E or L based on the observation that a 69% fragment of the genome was sufficient for cell transformation *in vitro* and was hence defined as the early region by analogy with SV40. However, within the early region, the E4 gene is now known to be expressed primarily within the late temporal class of mRNA. Primary transcripts can undergo differential splicing to produce multiple mRNAs (not shown), that in some cases fuse parts of reading frames together. The region lacking open reading frames is known as the long control region (LCR). P_{89} etc., RNA polymerase II promoters; poly A, sites of mRNA polyadenylation; Ori, origin of DNA replication.

Fig. 9.6 Gene expression by adenovirus type 5. (a) The phases of gene expression. The numbers E1A, L1, etc. refer to regions of the viral genome from which transcription takes place. (b) A transcription map of the adenovirus 5 genome. The genome is represented at the center of the diagram as a line scale, numbered in kilobase pairs from the conventional left end, with rightwards transcription shown above and leftwards transcription below. Genes or gene regions are named in boldface. Promoters of RNA polymerase II transcription are shown as solid vertical lines and polyadenylation sites as broken vertical lines. VAI and VAII are short RNA polymerase III transcripts. Individual mRNA species are shown as solid lines, color-coded according to the temporal phase of their expression (panel a), with introns indicated as gaps. The protein(s) translated from each mRNA is indicated above or adjacent to the RNA sequence encoding it. Structural proteins are shown by roman numerals (major proteins: II, hexon; III, penton; IV, fiber; pVII, core; see Chapter 11). PT, 23K virion protease; DBP, 72K DNA binding protein; pTP, terminal protein precursor; Pol, DNA polymerase. (Reproduced with permission from Leppard, K. N. (1998) *Seminars in Virology* **8**, 301–307. Copyright © Academic Press).

Fig. 9.7 Gene expression in herpes simplex virus type 1 infection. The three successive phases of gene expression are denoted α, β, γ corresponding to immediate-early, early, and late (see text). Solid arrows indicate the flow of material; dashed arrows indicate regulatory effects on gene expression; + indicates activation; – indicates repression.

Fig. 9.8 Adeno-associated virus gene expression. The 4.6-kb genome is represented as a line scale, with mRNAs beneath it with 5′ → 3′ polarity from left to right. ITR, inverted terminal repeat (see Section 6.6). Encoded proteins are shown as colored boxes: blue – early; green – late. See Section 9.7 for further details.

Fig. 9.9 (a) The mRNAs and proteins synthesized in avian leukosis virus-infected cells. In nonavian retroviruses, the protease is encoded as part of Pol and so is only present in the Gag-Pol fusion protein (see text for details). (b) The location of retroviral proteins within the mature virion. The role of the protease in the maturation of retrovirus particles is considered in Section 11.7. Note that the complexes of SU and TM in the envelope are actually trimers of each protein.

Fig. 9.10 Hepatitis B virus genome map. The closed circular DNA is transcribed from four promoters (Pr); arrowheads indicate 3′ ends. For details of protein expression, see text. Pre-S1, pre-S2 are the designations given to the upstream portions of the S reading frame which, when added to the N-terminus of S, give rise to the longer L and M surface proteins. DR1, DR2 are repeated sequences shown to orient the map (see Fig. 8.5).

10 The process of infection: IIIB. Gene expression and its regulation in RNA viruses

Fig. 10.1 Genome organization of reovirus. Each reovirus genome segment encodes a single major protein, but post-translational modification leads to the functional protein. Segment S1 also encodes an additional protein σ1s. The proposed functions of the proteins are shown in Table 3.2.

Fig. 10.2 Electron micrographs showing nascent mRNA leaving reovirus cores via the spike proteins. (From Bartlett N. M., *et al.* 1974. *Journal of Virology* **14**, 315–326.)

Fig. 10.3 Structure of a capped RNA molecule. Note the 5′–5′ phosphodiester linkage. Bases 1, 2, and 3 can be any of the four nucleic acid bases. If the 2′ positions of the ribose of bases 1 and 2 are not methylated the structure is referred to as a cap 0 structure, if only the 2′-*O*-methyl group on the ribose of base 1 is present it is called a cap 1 structure, and if a 2′-*O*-methyl group is present on both bases 1 and 2 it is a cap 2 structure.

Fig. 10.4 Arrangement of the overlapping open reading frames of the reovirus S1 gene mRNA. The relative locations and protein products of the two ORFs are indicated. The numbers refer to the position of the first nucleotide of the relevant codon.

Fig. 10.5 Representation of the cleavages of the poliovirus polyprotein and the smaller products to yield mature viral proteins. Note that most poliovirus mRNA lacks the 5′ VPg protein present on virion RNA. 3C′ and 3D′ are produced by cleavage of 3CD at an alternative site to that producing 3C and 3D.

Fig. 10.6 Alphaviruses synthesize two mRNAs in the infected cell. The larger mRNA also acts as the genome; the smaller, 26S mRNA has the same sequence as the 3′ end of the genome. (a) The mechanism for production of the two mRNAs is shown. (b) The proteins encoded by each mRNA and the pattern of proteolytic cleavage that results in the generation of functional proteins.

Fig. 10.7 Control of gene expression in the coronaviruses mouse hepatitis virus. (a) The products of transcription of mouse hepatitis virus and the proteins encoded by each mRNA. The common sequence found at the 5′ end of the genome RNA and all mRNAs is shown in red. (b) Negative sense RNAs are produced by a novel mechanism in which the virus-encoded RNA polymerase copies the 3′ end of the genome RNA template and completes synthesis by "jumping" to finish copying the immediate 5′ end. The negative sense RNAs are then used to synthesize individual mRNAs. Each mRNA contains the same 5′ end sequence.

Fig. 10.8 The three primary types of virus RNA found in influenza virus type A infected cells are shown, with the key differentiating features indicated. All eight genome segments have the three forms. The genome RNA has conserved terminal sequences which are present in complementary form in the antigenome which is an exact replica of the genome segment from which it was copied. The mRNA contains additional sequences at the 5′ end not present in the genome template and the mRNA is terminated before the end of the template is reached at which time a polyA tail is added by reiterative transcription (Box 10.5).

Fig. 10.9 The process of transcription from influenza virus genome segments. The three virus proteins, PB2, PB1, and PA, act together (a) to remove cap structures together with 10–13 nucleotides from the 5′ end of host cell mRNA (PB2) and (b) use these as primers to initiate transcription (PB1) followed by (c) extension of the newly synthesized RNA (PA) to produce (d) an mRNA molecule.

Fig. 10.10 Splicing in influenza virus RNA: synthesis of mRNAs encoding M1 and M2 proteins from segment 7 and NS1 and NS2 proteins from segment 8 of influenza virus type A. The shaded areas represent the coding regions. The reading frames of the unspliced mRNAs are shown but only the first ORFs, encoding M1 and NS1 from segments 7 and 8, respectively are used in these mRNAs. Note that the two major products from each pair of mRNAs share a short common amino-terminal amino acid sequence but differ in the majority of the sequence. The M3 protein has not been detected in infected cells.

Fig. 10.11 The ambisense strategy of the S RNA segment of the arenavirus, lymphocytic choriomeningitis virus.

Fig. 10.12 Diagrammatic representation of the sequential transcription process of the rhabdovirus vesicular stomatitis virus (VSV). Transcription occurs in a sequential, start-stop process described in the text. mRNAs are separated by nontranscribed regions, the lengths of which are virus-specific. As the polymerase complex moves towards the 5′ end of the template fewer transcripts are produced.

Fig. 10.13 Arrangement of the overlapping open reading frames of the Sendai virus P gene mRNA and the various potential protein products. (a) The relative locations and protein products of the ORFs are indicated. The arrow shows the insertion site for nontemplated G residues at nucleotide 1183 in the mRNA. The altered reading frames leading to the novel carboxy termini of the V (b) and W (c) proteins are indicated in red. Initiation of translation at AUG (white arrow) codons and the ACG (black arrow) for the C′ protein are indicated. The numbers refer to the nucleotide positions of the first nucleotide of the relevant codon.

11 The process of infection: IV. The assembly of viruses

Fig. 11.1 Effect of pH and ionic strength on the formation of aggregates of TMV A protein.

Fig. 11.2 (a–c) Simple model for assembly of TMV. See text for details.

Fig. 11.3 The packaging site of 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. 11.4 The "travelling loop" model for TMV assembly. 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 (a). The loop intercalates between the two layers of subunits and binds around the first turn of the disc. On conversion to the lock-washer (b) the RNA is trapped. As a result of the mode of initiation the longer RNA tail is doubled back through the central hole of the rod (c), forming a traveling loop to which additional discs are added rapidly.

Fig. 11.5 Summary of the steps involved in the assembly of poliovirus.

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

Fig. 11.7 Diagram of the morphological changes in the head structure of bacteriophage λ. The procapsid I structure loses the scaffolding proteins to produce a procapsid II structure which in turn acquires the phage genome DNA to form a mature head.

Fig. 11.8 Sites of maturation of various enveloped viruses. F, fusion of a vesicle with a membrane.

Fig. 11.9 Morphogenetic changes seen in HIV particles following budding from the surface of the host cell. (a) Budding of an HIV capsid. (b) Mixture of both immature (arrows) and mature HIV particles. (c) Mature HIV virion with characteristic cone-shaped core. (Taken from Hunter, E. 1994. *Seminars in Virology* **5**, 71–83.)

12 The immune system and virus neutralization

Fig. 12.1 Summary of some of the responses of the immune system to viruses and virus-infected cells.

Fig. 12.2 Schematic relationship between the epitope (or antigenic determinant), antigenic site (a collection of overlapping epitopes), and antigen. BCRs can recognize almost any chemical type of molecule, but TCRs react only with peptide epitopes. Each epitope shown is unique.

Fig. 12.3 Schematic relationship of the compartmentation of the systemic and mucosal immune systems.

Fig. 12.4 Generalized immunoglobulin molecule, showing (a) the outline structure, consisting of two identical dimers formed of H–L polypeptides. Note the sequence-variable (V) region of the H and the L chains. Each contains three hypervariable sequences of 10–20 residues (not shown) that are folded together to form the unique epitope-binding site (paratope). The remaining H and L sequences are relatively constant. The constant region is subdivided into domains with sequence homology (C_H1 to C_H3). IgM and IgE both have an additional domain (C_H4). The molecule is also divide into an N-terminal half that binds epitope (Fab), and a C-terminal half (Fc) that is reactive with various cell mediators. IgM and IgA form 5-mers and 2-mers respectively linked by their C ends with a joining (J) polypeptide. (b) The globular domains, with arrows indicating the flexibility which allows the molecule to bind to two (identical) epitopes, which can be different distances apart in three dimensions. SS, disulfide bond.

Fig. 12.5 Hypothetical scheme based on the length of the cell receptor to explain inhibition by antibody of attachment of virus to cell B but not cell A.

Fig. 13.1 An acutely cytopathogenic infection of a HeLa cell line by poliovirus, a picornavirus. Cells were inoculated with an MOI (multiplicity of infection) of 10 infectious units per cell, so that nearly all cells were infected and a one-step growth curve results. Note that intracellular infectivity declines at later times as cells die.

Fig. 13.2 Different types of infection caused by simian virus 5 in BHK (baby hamster kidney) cells and MK (monkey kidney) cells. (a) The acutely cytopathogenic infection in BHK cells. Note that virus yield drops after 24 hours. (b) The initial one-step growth curve in MK cells which kills no cells and becomes persistent. (c) The cumulative yield from MK cells infected in (b) over 30 days. Cells grow normally during this infection and have to be subcultured at intervals of approximately 4 days.

Fig. 13.3 A persistent infection established between a virus that normally causes an acutely cytopathogenic infection, and defective-interfering virus. The dynamic cycles of production of infectious and DI virus eventually give way to a low-level steady state persistent infection.

Fig. 14.1 Diagram to show the three-way interactions that decide the outcome of infection.

Fig. 14.2 A representation of an experimental infection in humans caused by a rhinovirus following deliberate intranasal inoculation.

Fig. 14.3 The risk of becoming a carrier who is chronically infected with hepatitis B virus (HBV) depends on the age at which a person is infected.

Fig. 14.4 The relationship between virus infection and tumorigenesis.

15 Mechanisms in virus latency

Fig. 15.1 Formation of circles of phage λ DNA after infection. The base composition of the complementary cos regions are shown. In λ these are extensions to the 5′ ends of the genome.

Fig. 15.2 The circular map of the bacteriophage λ genome. The genes involved in the lytic cycle are indicated. The positions of the phage promoters and transcriptional terminators involved in the lytic cycle are shown together with the mRNAs produced at immediate early, early, and late times. The sequence attP is relevant to lysogenic replication (Section 15.3).

Fig. 15.3 The region of the bacteriophage λ genome encoding the genes responsible for lysogeny. (a) The cII protein activates the P_{RE} and P_{int} promoters to produce mRNAs for the cI and Int proteins, respectively. Note the mRNA from P_{RE} contains sequences antisense to the *cro* gene. (b) The cI protein binds O_L and O_R, inactivating P_L and P_R, respectively, while activating P_{RM}, ensuring its own continued synthesis throughout lysogeny. The Cro protein also inhibits P_L and P_R activity. (c) The steady state of *cI* gene expression during lysogeny.

Fig. 15.4 (a) Proposed model for the insertion of phage λ DNA into the bacterial genome by reciprocal recombination between phage and host DNA. (b) The common sequence in the bacterial and λ attachment sites.

Fig. 15.5 Molecular control of latency in herpes simplex virus type 1 infection. Of 72 viral genes, only the latency-associated transcripts (LATs) are synthesized during latency. External factors that can break the latent state upregulate the synthesis of the viral nonstructural protein, ICP0, which then enhances its own expression. This activates expression of the α genes and unlocks the pathway to acute infection (see text).

Fig. 15.6 Development and resolution of a cold sore from reactivated HSV-1. On day 1, reactivated virus is released from cells of the dorsal root ganglion and transported down the sensory neuron. It then infects adjacent epithelial cells. Expression of major histocompatibility complex (MHC) I protein complexed with viral peptide on the cell surface is inhibited (see text) and virus-specific memory $CD8^+$ T cells are not activated. NK cells and virus-specific $CD4^+$ T cells move gradually into the lesion and release cytokines. By day 2, they are releasing enough γ-interferon to upregulate the expression of MHC class I protein. The $CD8^+$ memory T cells are then activated and virus-infected cells are cleared. (From Posavad *et al.*, 1998. *Nature Medicine* **4**, 381.)

16 Transmission of viruses

Fig. 16.1 Route taken by aerosol droplets containing virus particles that are breathed into the respiratory tract. Note the defences designed to trap and remove all sorts of small particles: turbinate baffles, the mucociliary flow that runs counter to inspired air, and macrophages. The airways become progressively narrower (here much foreshortened) and end in alveoli, each of which is formed by a single cell. An alveolar macrophage can be seen in one of the alveoli. (Adapted from Mims, C. A., White, D. O. 1984. *Viral Pathogenesis and Immunology.* Blackwell Scientific Publications, Oxford.)

Fig. 16.2 Examples of zoonoses. In (a) the fox vector is the primary host. Rabies can be passed on infected saliva by biting and also, but more rarely, by the respiratory route. Biting arthropods are the vectors in (b) and (c), and the virus multiplies in both the vertebrate and the arthropod. In (b) monkeys are the primary host and yellow fever virus is spread by the female mosquito (the male mosquito does not feed). The primary host of sandfly fever virus (c) may be the gerbil, but as shown here humans can be the reservoir when there is a high incidence of infection. In these examples, virus does not pass from person to person.

17 The evolution of viruses

Fig. 17.1 Animal species that are naturally infected with influenza A viruses. Wild birds of the sea and shore form the natural reservoir (top). Known routes of transmission are indicated by continuous arrows and probable routes of transmission by broken arrows.

Fig. 17.2 History of antigenic shifts of influenza A viruses in humans. The outer circle denotes the year of emergence of a new subtype that is shown in the inner circle, the duration of the reign of that subtype, and when it is replaced by another subtype. A time scale is shown in the middle circle. Approximate worldwide mortality figures for each shift are indicated in millions. The 1900 shift did not cause a serious pandemic. Currently (2006) H3N2 and H1N1 subtypes coexist. Some occasional infections of people with bird or pig viruses that are not transmitted person to person are noted in the inner circle (but see text).

Fig. 17.3 Summary of the events leading up to an antigenic shift of human influenza virus. The evolutionary time scale is not known but probably takes several years.

Fig. 17.4 Recombination (reassortment) between an existing human influenza A virus (H2N2) and a new virus from the wild bird reservoir (H3N*x*, where *x* represents an unknown neuraminidase subtype; see text) that gives rise to antigenic shift. The two viruses simultaneously infect a cell in the respiratory tract, and the eight genome segments from each parent assort independently to progeny virions. The example shows a novel progeny virion (H3N2) that comprises the RNA segment encoding the H3 avian hemagglutinin and the seven remaining segments from the existing human virus.

Fig. 17.5 Diagram showing antigenic drift of type A influenza virus in humans. This could represent either the HA or NA. Each point is a virus strain isolated in a different year.

Fig. 17.6 Diagram showing the course of antigenic shift and drift of influenza A viruses in man. The first virus, isolated in 1933, was H1N1. This arose by antigenic drift from the 1918 virus. Other shift viruses appeared in 1957 (H2N2) and 1968 (H3N2). A 1950 H1N1 virus reappeared in 1977. Drift is shown schematically. The 1957 N2 was acquired by the H3N2 shift virus, and has drifted from 1957 to the present day.

Fig. 17.7 Model of antigenic drift of influenza type A and type B viruses. Points on the same level represent drift variants that arise in the same year. The branch length indicates the relative change in antigenicity from virus in the preceding year. Drift is shown for an arbitrary 7-year period. See text for further discussion.

Fig. 17.8 "Antigenic drift" in the laboratory: a single neutralizing monoclonal antibody can select a population of influenza virus escape mutants that is no longer neutralized by the selecting MAb. Another round of selection with a second MAb produces virus that now carries two amino acid substitutions, but selection with the two MAbs simultaneously is completely neutralizing (not shown).

Fig. 17.9 An epitope-biased serum antibody response in a rabbit injected with influenza A virus. Nearly all the HA-specific antibody is accounted for by the response to a single epitope in antigenic site B. (From Lambkin, R. and Dimmock, N. J. 1995. *Journal of General Virology* **76**, 889–897.)

Fig. 17.10 How antigenic drift may occur in nature, bearing in mind that clinically significant drift viruses (that cause epidemics) have four or more amino acid residues changed in two or more antigenic sites present on the hemagglutinin protein. Note that people with biased antibody responses may be uncommon and that there may be any number of nonselective infections of other individuals occurring between the four persons shown here, as indicated by the broken arrow of transmission. A similar process could cause drift of the NA protein.

Fig. 18.1 Monthly incidence of respiratory syncytial virus infection in the UK showing pronounced annual epidemics peaking in November–January.

Fig. 18.2 Three scenarios for the relationship between the route of entry of a virus, its circulation in the body, site of replication, and sites of disease manifestation. (a) Virus enters and circulates widely but can only infect cells in one location, where it replicates and causes disease, e.g hepatitis B virus, with entry directly into the blood or by sexual contact and replication in the liver. (b) Virus infects cells at the portal of entry, where it replicates causing signs and symptoms, but cannot spread elsewhere despite its use of a cell receptor that is widely distributed, e.g. rhinovirus, respiratory syncytial virus with entry and exit via the respiratory tract. (c) Virus infects cells at the portal of entry, where it replicates and then spreads systemically to give a variety of signs and symptoms, e.g. measles virus, which again enters and exits via the respiratory tract. Blue: movement of infecting virus; red: movement of progeny virus.

Fig. 18.3 (a) Section from the ileum of a piglet 72 hours post-infection with rotavirus. (b) Section from the ileum of a control, mock-infected, piglet. Note the shortening of the villi and the absence of vacuoles (intracellular white areas) in the villous cells in (a) as compared with (b). These are indicative of normal active absorptive function. The bar represents 50 μm. (Adapted from Ward, L. A., Rosen, B. I., Yuan, L., Saif, L. J. 1996. *Journal of General Virology* **77**, 1431–1441, with permission from the Society for General Microbiology.)

Fig. 18.4 An example of lung consolidation caused by respiratory syncytial virus infection in an immunosuppressed patient. The lungs have been imaged by high resolution CT scan. There are nodular lesions in the right lung (R, arrows) while the left lung (L) shows areas of consolidation (white) where no air is present. From Escuissato, D. L. *et al*. 2005. *American Journal of Roentgenology* **185**, 608–615. (Reprinted with permission from the *American Journal of Roentgenology*.)

Fig. 18.5 A representation of the flows of blood and bile to and from the human liver. Oxygenated blood arrives via the hepatic artery while the bulk of blood flow into the liver comes from the gut via the portal vein, supplying nutrients for metabolism. Blood returns to the circulation via the hepatic vein. One of the products of the liver is bile, which is stored in the gall bladder and delivered to the gut via the bile duct.

Fig. 18.6 A simplified diagram of a polarized epithelium showing virus exiting an infected epithelial cell either (1) via its apical surface to reach the lumen of the organ, or (2) via the basolateral surface to reach the vascular system. The apical and basolateral surfaces of the epithelial cells are separated by tight junctions that connect the cytoplasm of adjacent cells and prevent movement of membrane proteins between the two surfaces. Not to scale. See Section 18.8 for further details.

19 HIV and AIDS

Fig. 19.1 Dendrogram showing the relatedness of some major lentiviruses. BIV, bovine immunodeficiency virus; CAEV, caprine arthritis-encephalitis virus; VMV, visna-maedi virus; EIAV, equine infectious anemia virus; SIV, various strains of simian immunodeficiency virus (SIV_{cpz} was isolated from a chimpanzee); HIV, various strains of human immunodeficiency virus type 1 and 2; HTLV-1, human T cell lymphotropic virus type 1. (Courtesy of Georg Weiller.)

Fig. 19.2 Global distribution of HIV-1 clades, recombinants, and circulating recombinant forms (CRF). The 10 different epidemic patterns are indicated by different colours. HIV-2 accounts for only a minority of infections, mainly in West Africa but now also in India. (From F. E. McCutcheon and IAVI, 2003. http://www.iavi.org/iavireport/0803/clades-vax.htm)

Fig. 19.3 The positive sense ssRNA genome of HIV. The scale indicates the genome size in nucleotides. Note that all three open reading frames (ORFs) are used, but only simultaneously in the region of nucleotide 8500. *Gag*, *pol*, and *env* are transcribed and translated to give polyproteins which are then cleaved by proteases to form the virion core proteins, enzymes, and spike proteins as shown. The nomenclature "p17" indicates a polypeptide having an M_r of 17,000, etc. Genes not aligned require a frame shift for expression. Tat and Rev proteins are expressed from spliced RNAs.

Fig. 19.4 Regulation of HIV-1 gene expression from provirus DNA integrated into the cellular genome. This occurs in two phases: early expression of regulatory genes and late expression of structural genes. (From Cullen, B. R. 1991. *Journal of Virology* **65**, 1053.)

Fig. 19.5 An HIV-1 virion with the major proteins identified (a). There are others present (see text). (b) A monomer of the trimeric envelope spike protein adapted from the structures suggested by Leonard *et al.* 1990. *Journal of Biological Chemistry* **265**, 10373) and Gallagher (1987, *Cell* **50**, 327). Each circle represents an amino acid residue. The gp160 has been cleaved to form gp41 (open circles), the COOH-terminal transmembrane anchor, and the distal gp120 (solid circles). gp120 contains five antigenically variable (V) regions and five intervening conserved (C) regions; it has several intramolecular disulfide bonds, and is heavily glycosylated. gp41 is also glycosylated (not shown here). (c) An alternative scheme for the C-terminal transmembrane and tail regions of gp41. This has three transmembrane (tm) domains and a 100-residue intravirion tail; the N-terminal tm domains are formed by the same residues that comprise the single tm region in (b); tm 2 and tm 3 support 41 residues, the minor ectodomain, which expresses several epitopes (gray), including one that is neutralizing (adapted from Hollier, M. J., Dimmock, N. J. 2005. *Virology* **337**, 284). The major N-terminal gp41 ectodomain and gp120 are not shown.

Fig. 19.6 Model of a gp120 monomer of HIV-1 based on the atomic structure determined by Kwong *et al.* (1998, *Nature, London* **398**, 648). (a) A simplified skeleton structure, and (b) with V loops added. The model is orientated with gp41 and the virus membrane at the top of the page. The monomers meet in a trimer and obscure the inner face. Much of the outer face is obscured with carbohydrate. Only a small proportion of the gp120 surface has epitopes that interact with neutralizing antibody (see text), although there are also neutralizing epitopes on gp41. There is also an atomic structure of an isolated gp41 ectodomain, but it is not known how it interacts with gp120. (From Burton, B. R., Parren, P. W. H. I. 2000. *Nature Medicine* **6**, 123.)

Fig. 19.7 A case study of AIDS, which ended in death in year 9. The $CD4^+$ T cell loss was exceptionally rapid. This case occurred before anti-HIV therapy was available. PGL, persistent generalized lymphadenopathy; CMV, cytomegalovirus. A, B, and are defined in Box 19.4.

Fig. 19.8 The course of HIV-1 infection, the inverse relationship betweeninfectious virus load and T cell concentrations in plasma. A, B, C refer to the categories of infection (see Box 19.4 and text).

Fig. 19.9 The dip-stick test for antibody to HIV.

Fig. 19.10 Comparison of the formulae of AZT (3′-azido-2′,3′-dideoxythymidine) and deoxythymidine, and the formulae of some other nucleoside analog inhibitors of HIV DNA synthesis. Note that none of the analogs shown have a hydroxyl group in the 3′ position (green). Thus they cannot form a bond with the next nucleotide, and act as chain terminators. All these molecules require phosphorylation by cellular enzymes before being incorporated into DNA. See text for commercial names.

Fig. 20.1 Alternative scenarios for multistep progression from a normal to a cancer cell occurring by the acquisition of genetic alterations, sometimes including the effect of specific virus genes. Genetic alterations are symbolized by arrows, though the number of arrows shown is not intended to indicate the actual number of events involved in converting a normal cell into a fully malignant cell. The specific nature of early events in the pathways probably predisposes cells to suffer further mutations.

Fig. 20.2 Demonstration of cooperation between gene functions to achieve full oncogenic transformation. While the E1A region alone of adenovirus type 12 is transforming, the cells do not survive indefinitely (abortive transformation). Only cells carrying the adenovirus type 12 E1B gene as well are fully transformed and tumorigenic in syngeneic rats. The E1B gene alone gives no transformation. BRK, baby rat kidney.

Fig. 20.3 The transforming proteins of SV40, polyoma, papilloma, and adenovirus. Horizontal green bars represent the proteins indicated, not to scale. The adenovirus E1A 13S and 12S polypeptides are highly related, differing only in the removal (by splicing of the mRNA) of 46 amino acid residues from the shorter protein. For expression and sequence relatedness of the SV40 and polyoma proteins, see Section 9.2. The various colored blocks indicate functional rather than sequence relatedness, although there is sequence similarity between the indicated Rb-binding sequences. Red: Rb-binding; blue: p53 binding; yellow: accessory region required to release transcription factors from bound Rb; grey: Src protein binding. For definitions of Rb, p53, and Src, see text.

Fig. 20.4 Models of retrovirus oncogenesis involving integration of viral genomes into cellular DNA. (a) Integration of a defective viral genome carrying an oncogene whose transcription is under the control of the strong promoter in the viral LTR. (b) Integration of a virus that lacks an oncogene, so that its right-hand LTR drives expression of a cellular proto-oncogene (proto-onc). (c) Expression of a transcription-enhancing product from viral gene X which affects both viral and cellular transcriptional control, upregulating expression of a proto-oncogene.

Fig. 20.5 Four variations on the retroviral insertional mutagenesis model shown in Fig. 20.4b. Integrated proviruses are indicated in brown. Transcriptional regulation of a cellular gene will be altered by the provirus in all four scenarios, because the enhancers in each LTR operate in a position- and orientation-independent manner to upregulate transcription from the gene's endogenous promoter.

21 Vaccines and antivirals: the prevention and treatment of virus diseases

Fig. 21.1 Exponential kinetics of virus inactivation. Note the "resistant" fraction which is inactivated more slowly.

Fig. 21.2 Newspaper advertisement aimed at increasing public awareness and acceptance of vaccination against rubella virus.

Fig. 21.3 Sequence changes occurring (a) when the original type 3 poliovirus wild-type neurovirulent Leon strain was attenuated to form the Sabin vaccine, and (b) when the vaccine strain reverted to the neurovirulent 119 isolate. (Drawn from information in Almond, J. W. 1987 *Annual Review of Microbiology* **41**, 153–180.)

Fig. 21.4 Diagram of an influenza A virus hemagglutinin spike deduced from X-ray crystallographic analysis. The spike is composed of three identical monomers. Only when antibody binds to the red areas is the virus neutralized. The attachment site that binds to *N*-acetyl neuraminic acid on the host cell is in black.

Fig. 21.5 Antibody epitopes constructed in various ways from segments of polypeptide sequence represented by an arbitrary two-letter code and viewed by the immune system in the direction arrowed.

Fig. 21.6 Identification of hydrophilic regions (putative epitopes) of a protein by calculating the hydropathic value of each segment of amino acid sequence across a moving window of seven residues, i.e. the amino acid in question and the three residues on either side.

Fig. 21.7 Construction of an infectious vaccinia virus recombinant expressing the influenza virus hemagglutinin. BUdR (bromodeoxoyuridine) inhibits DNA synthesis.

Fig. 21.8 Coverage of measles vaccination of infants around the world, 2004. (From WHO/UNICEF.)

Fig. 21.9 Fall, rise, and fall again in the number of reported cases of measles in the USA following vaccination, 1960–2005. Note change of scale in the insets. About one-third of the cases in the last few years have been contracted outside the USA and imported by visitors or returning residents.

Fig. 21.10 Progress of poliovirus eradication worldwide, showing the countries in which virus was endemic in 1988 (top) and in 2004. (Modified from the WHO Expanded Program on Vaccination.)

Fig. 21.11 The formula of the chain-terminating nucleoside, aciclovir. Note that most of the cyclic sugar ring is missing – hence the name. The hydroxyl group shown has to be phosphorylated before aciclovir can be incorporated into nascent DNA.

Fig. 21.12 Aciclovir is toxic for herpes simplex virus-infected cells but not noninfected cells

Fig. 21.13 (a) Induction of interferons-α/β (IF) by viral double-stranded (ds) RNA. The dashed arrow indicates that we do not know if dsRNA is required to enter the nucleus to initiate interferon mRNA synthesis or if it acts through an intermediate. (b) Binding interferon by nearby cells creates in them an antiviral state through inhibition of protein synthesis – either from the phosphorylation that results from the induced protein kinase (PKR), or by stimulating 2′,5′-oligoadenylate synthetase and ribonuclease (RNase) L.

Fig. 21.14 Action of interferons-α/β: activation of RNase L. Inset: the formula of the 2′,5′-A trinucleotide.

Fig. 21.15 Successful interferon-α treatment of a patient with a chronic hepatitis B virus (HBV) infection. Note the fall in viral DNA, viral DNA polymerase, and the viral antigens HBeAg and HBSAg. These all became undetectable, showing that the infection had been cleared. The transient rise in aspartate aminotransferase (AST), a marker of liver damage, signifies the destruction of HBV-infected liver cells by virus-specific $CD8^+$ CTLs following the upregulation of MHC class I antigens (see text). (From Thomas 1990. *Control of Virus Diseases. Society for General Microbiology Symposium 45*, pp. 243–259. Ed. N. J. Dimmock, P. D. Griffiths, & C. R. Madeley. Cambridge: Cambridge University Press.)

Fig. 22.1 Spongiform degeneration and protein (PrPsc) plaque deposition in CWD-infected elk brain. The images show sections from brain cortex either hematoxylin & eosin stained (A) or with detection of PrPsc deposits by immunohistochemistry (brown stain, B–D). Magnifications: A, ×180; B, C, ×280; D, ×720. (Reproduced with permission from Liberski P. P. *et al.* 2001. *Acta Neuropathologica* **102**, 496–500. © Springer-Verlag.)

Fig. 22.2 A model for the propagation of TSE infectivity according to the prion hypothesis. PrPc (circles) and PrPsc (squares) represent the normal and an abnormal conformation respectively of the *prnp* gene product. Host PrP in the normal and abnormal conformation are pale and dark blue respectively. The initiating infectivity (red) is proposed to have the same structural features and properties as the progeny PrPsc molecules (although see discussion of the species barrier, Section 22.4). Steps 2 and 3 in the propagation cycle may be reversible. Alternative models differ principally in suggesting that polymerization of the altered structural form is important to its potential for catalyzing further structural conversions.

Fig. 22.3 The incubation time to disease of five distinct, mouse-adapted, scrapie strains upon serial passage in hamsters. Each colored line represents data from a different scrapie strain. Passage 1 shows a longer and more variable incubation time for each strain because of the species barrier to transmission (Section 22.4). Subsequent passages show remarkably constant, but strain-specific, incubation times. Standard errors, except where shown, were insignificant. (Data are taken from Kimberlin, R. H. *et al.* 1989. *Journal of General Virology* **70**, 2017–2025.)

Fig. 22.4 The interaction between pathogenic mutations at position 178 of human PrP and the amino acid present at the polymorphic position 129 on either the mutant or normal allele. Both the nature of the disease pathology, its time of onset, and rate of progression are determined by the position 129 polymorphism. Blue: regions of spongiform degeneration; brown: regions of neuronal loss and replacement with astrocytes and glial cells. See Box 22.3 for further details. (Redrawn, with permission, from Gambetti, P. 1996. *Current Topics in Microbiology and Immunology* **207**, 19–25. With kind permission of Springer Science and Business Media.)

Fig. 22.5 Multiplication of the scrapie agent in the brain and spleen of mice infected peripherally and the time course of disease.

Fig. 22.6 Annual incidence of BSE in the UK cattle herd, 1987–2005. (Graph drawn from data taken from the UK Department for Environment, Food and Rural Affairs (DEFRA) website.)

Fig. 22.7 The biological and molecular properties of different BSE isolates transmitted to mice are very similar to each other, and to those of vCJD isolates. (a) The incubation times of BSE isolates from cattle upon transmission to mice. Each line on the figure represents a different BSE isolate and the four types of symbol show the mean incubation time for that isolate in four different strains of mouse. (Adapted, with permission, from Bruce, M. E. *et al.* 1997. *Nature* **389**, 498–501. Reprinted by permission from Macmillan Publishers Ltd.) (b) Molecular signatures of TSE isolates. A schematic representation of an immunoblot analysis of polypeptides from the brains of mice infected with different TSE isolates that have been separated by SDS–PAGE. The bands represent protease-resistant core fragments of PrPsc, differing in length and glycosylation pattern, that have been detected with antibody to PrPsc. The thickness of each band represents its intensity in the original analysis. Human PrP types 1, 2, and 3 are seen in various sporadic and iatrogenic CJD isolates; the type 4 pattern is seen in variant CJD isolates and is very similar to the pattern consistently generated by BSE isolates in this type of analysis. (Reprinted from *Current Opinion in Genetics and Development* vol. 9, J. D. F. Wadsworth, G. S. Jackson, A. F. Hill & J. Collinge, Molecular biology of prion propagation, 338–345 (1999) with permission from Elsevier, using original data from Collinge, J. *et al.* 1996. *Nature* **383**, 685–690.)

Fig. 23.1 (a) Scheme outlining the random combinatorial method for the cloning of human antibody single-chain Fv fragments. Its success lies in the ability to select rare recombinant phages expressing the required antibody activity (about 1 in 10^6 phages). (b) Construction of the fd phagemid containing the fused V_L–linker–V_H gene. This is transformed into *E. coli*. The bacterium is then infected with phage (fd) and progeny phages are produced, each having a single recombinant V_L–linker–V_H polypeptide displayed on its surface (i). This polypeptide folds to form a functional antibody-binding site (ii). The "phage antibody" requires just one molecule of nonmodified attachment protein (iii) to be infectious.

Fig. 23.2 (a–d) Production of recombinant retroviruses by transfection of cloned recombinant genome plasmid into a packaging cell line. Cells may be cultured on as a producer cell line, with recombinant virus being harvested from the growth medium. The essential retroviral protein coding genes, *gag*, *pol*, and *env* (see Section 9.8) are integrated in the packing cell line in two distinct segments, to minimize the risk that recombination between these sequences and the vector could produce a viable retrovirus again.

Fig. 23.3 The genomes of adenovirus gene therapy vectors, either (a) deleted for the essential E1A and E1B genes, plus nonessential E3 genes (to make more space for the transgene), or (b) deleted for all adenovirus sequences except the termini, which provide origins of replication and the signal for packaging the genome into particles. In (a), the maximum capacity for the transgene is around 7 kbp, while in (b), the maximum is around 35 kbp and, for short transgene sequences, additional "stuffer DNA" is needed to achieve the minimum genome length necessary for stable particle formation. The viral genes are shown in simplified outline in (a), with early genes colored red and late genes colored purple (see Section 9.4). Viral sequences deleted during vector construction are shown as brown boxes in the genome and the affected genes are shown striped in (a).

Fig. 23.4 Eggs of the ichneumonid wasp only develop in their host caterpillar when injected together with a polydnavirus.

Fig. 23.5 Influenza A virus NS1 protein blocks the interferon (IFN) response on two levels: (a) virus infects cell and viral NS1 binds viral dsRNA and inhibits the production of type I interferons-α and -β; (b) virus infects cell and NS1 prevents the manifestation of the antiviral state in this cell by inhibiting protein kinase R (PKR). NS1 does this by activating the $P58^{IPK}$-mediated host stress response, by interacting directly with PKR, and by interfering with interferon response factors (IRFs). ISGs, interferon-stimulated genes; JAK, Janus kinases; STAT, signal transducer and activator of transcription; IF-2α, eukaryotic initiation factor-2α. Blocked lines represent inhibition. (Adapted from Katze, M. G. *et al.* 2002. *Nature Reviews Immunology* **2**, 675–687.)