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7 Virus–cell interactions

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KEY POINTS

- Viruses are completely dependent on the host cell for their replication.
- A part of the *capsid* (in the case of *non-enveloped viruses*) or *envelope* (in the case of *enveloped viruses*) binds to a specific receptor or receptors on the host cell to initiate entry of the virus into the cell.
- The interaction of viruses with cells can result in:
  - *production* of new virus particles with or without lysis of the host cells
  - *abortive infection*
  - *latency*
  - *transformation.*
- In the productive replication cycle, the sequential stages are *attachment* (adsorption), *entry* (penetration) into the cytosol, *uncoating*, synthesis of *viral macromolecules* (mRNA, proteins and genomes), *assembly* of new viral particles, *morphogenesis* and *release*.
- In latency, the virus persists as its genome, with limited expression of selected viral genes, sometimes as RNA only.
- In viral transformation, the virus persists as its genome, with expression of selected viral proteins that induce the host cell to behave like a tumour cell.

Viruses are totally dependent on the cells they infect to provide the energy, metabolic intermediates and most (in some cases all) of the enzymes required for their replication. With advances in the techniques of molecular virology, crystallography and modelling, together with the classical methods of electron microscopy, titration and biochemical assay, it has become possible to study virus–cell interactions to a sophisticated level. The picture that has emerged, and is still emerging, is a fascinating one, as viruses are found to associate with, and affect, cells in a wide variety of ways. The range of possible interactions is indicated in Table 7.1. It is possible to divide these into three broad categories that show considerable overlap:

1. Viruses that infect and replicate within cells causing the cells to lyse when the progeny virions are released. This is called a *cytolytic cycle*; the infection is productive and the cell culture demonstrates *cytopathic effects*, which are often characteristic of the infecting virus. The host cells are termed *permissive*. In some instances viruses are produced from the infected cells but the cells are not killed by the process: that is, the infection is *productive* but *non-cytolytic*, and may become *persistent.*
2. Viruses that infect cells but do not complete the replication cycle. The infection is thus called *abortive* or *non-productive*. Abortive infections can be due to a mutation in the virus so that some essential function is lost, or to the production of defective interfering particles, or to the action of interferons. It may be possible to manipulate the conditions in vitro to obtain a *steady state* or *persistent* infection in which infected and uninfected cells coexist and there is some, generally limited, virus production.
3. Viruses that enter cells but are not produced by the infected cell. The virus is maintained within the cell in the form of DNA, which replicates in association with the host cell DNA. The host cell is termed *non-permissive* and the infection is *non-productive*. Occasionally this type of interaction results in *transformation*, where the cell exhibits many of the properties of a tumour cell. In other cases a *latent infection* ensues in which little or no viral gene expression is found, no viral replication occurs and the cell retains its normal properties.

Table 7.1 The range of virus-cell interactions

Type of infection/effect on cells	Comment
Cytolytic	Virus produced
Non-cytolytic (persistent)	Virus produced
Abortive	Virus not produced
Abortive (persistent)	Virus produced
Latency (persistent)	Viral nucleic acid present
Transformation (persistent)	Viral nucleic acid present

THE CYTOLYTIC OR CYTOCIDAL GROWTH CYCLE

Although there are large differences in the details of the lytic growth cycle depending on the virus studied, and to some extent on the host cell, certain features are common, and a simplified description is given first. The quantitative aspects of virion production were determined initially using bacteriophages, but have now been ascertained for many animal viruses growing in vitro in cell culture. A one-step growth curve is obtained when samples are removed from an infected cell culture at intervals and assayed for the total content of infectious virus after artificial lysis of the cells (Fig. 7.1).

In the early part of the cycle, virus particles come into contact with the cells, and may then *attach* or *adsorb* to them. This marks the start of the *eclipse phase*. The virion then *enters* or *penetrates* into the host cell and is partially *uncoated* to reveal the viral genome. *Macromolecular synthesis* of viral components follows. This can often be divided into *early* and *late phases* separated by the replication of the viral nucleic acid. Early messenger RNA (mRNA) is first transcribed and translated into proteins. These are frequently non-structural proteins and enzymes required to undertake nucleic acid synthesis and the later stages of replication. Viral nucleic acid is then produced, followed by late mRNA transcription and translation. Most proteins synthesized at this stage are structural ones, and will make up part of the final virion. The eclipse phase ends with the *assembly* and *release* of newly formed virus particles. The cycle is shown in diagrammatic form in Figure 7.2, and can vary from as little as 8 h for some picornaviruses to more than 40 h for human cytomegalovirus, a herpesvirus.

Attachment (adsorption)

The initial interaction is by random collision and depends on the relative concentrations of virus

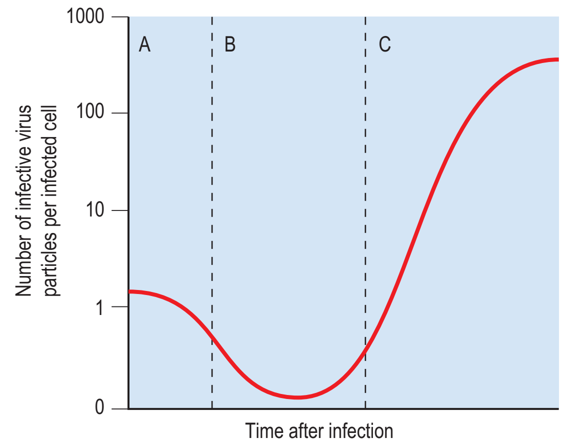

Fig. 7.1 Lytic growth cycle of a virus. Samples are removed from the infected culture at intervals and assayed for the total content of virus. Phase A, adsorption; phase B, eclipse; phase C, assembly and release.

particles and cells. Under in vitro conditions, the ionic composition of the culture medium is an important factor as both viruses and cells are negatively charged at neutral pH and thus tend to repel one another. The presence of cations, such as Mg^{2+}, helps to promote close contact. Adsorption then takes place through *specific binding sites* on the virus and *receptors* on the plasma membrane of the cell. It is largely a temperature- and energy-independent process. Viruses vary widely in the range of cells to which they can adsorb, depending on the nature of the sites to which they attach and how widespread they are among cells of different types, tissues and species. The presence of the receptor determines whether the cell will be susceptible to the virus, but the cells must also be *permissive*: that is, for successful production of new virions, they need to contain the range of intracellular components required by the virus for its replication. The ability of a virus to enter and replicate in a particular cell type is called tissue or cell *tropism.*

Many cellular receptors are protein in nature but they can also be composed of carbohydrate or lipid. There is considerable interest in identifying receptors for particular viruses, as the attachment step is a potential target for antiviral therapy and could aid in the understanding of viral pathogenesis. Specific receptors for selected viruses have been described, but have frequently been disputed. Newer techniques involving monoclonal antibodies, molecular cloning and gene transfer have helped to resolve these issues. It has become apparent that more than one type of receptor molecule may be required by the majority of viruses to complete the entry stage of the replication cycle. Indeed it is likely that there is a complex

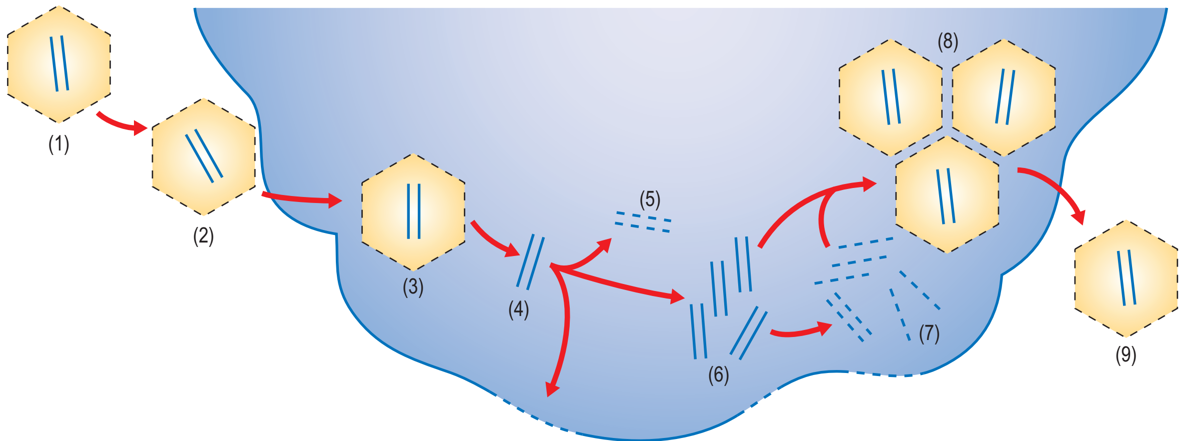

Fig. 7.2 A simplified viral replication cycle showing a hypothetical virus particle (1) attaching to the surface of a susceptible cell (2), entering into the cell (3), being uncoated (4), undergoing early transcription and translation (5), then replication of the viral nucleic acid (6), late transcription and translation (7), and, finally, assembly of new virus particles (8) and release from the cell (9).

interaction between different functional domains of the virus and several receptor arrays. First there is the 'true' attachment step whereby the virus binds to the cell receptor, and then entry itself may involve a further set of receptors called *co-receptors* or *post-binding receptors*, acting either in succession or in parallel. These interactions frequently induce conformation changes in the surface proteins of the virus, exposing hidden domains that are required for the entry step (see next section). This more complicated view of the initial contact between the virus and the cell suggests that the binding receptor may not be the only determinant of tropism, and the adsorption process may be influenced by factors such as virus strain, cell type and even the multiplicity of infection.

In Table 7.2, examples of viruses whose cellular receptors have been identified are shown.

Entry (penetration)

Entry occurs immediately after attachment and, unlike adsorption, requires energy and does not occur at 0°C. The speed of this stage of the replication cycle varies between different viruses, some penetrating into cells in less than a second and others taking several minutes. In addition, the efficiency of the process varies from 50% of attached viruses entering successfully to less than 0.1%. Entry is complex and, despite much study, it is still not clear exactly what the steps are for the majority of viruses.

For viruses with envelopes, penetration is accomplished by membrane fusion catalysed by fusion proteins in the viral envelope. The fusions proteins that mediate entry have been divided into two categories. *Class I fusion proteins* include influenza haemagglutinin protein, human immunodeficiency virus (HIV) gp120 and paramyxovirus fusion protein. They are all cleaved into two pieces during synthesis, are found as trimeric spikes protruding from the surface of the viral particles and contain a fusion peptide characteristically composed of 20 hydrophobic amino acids. In some cases, for example HIV, fusion occurs at the cell surface at neutral pH with the activation energy being provided by receptor binding. In other cases, for example influenza virus, receptor-mediated endocytosis occurs. Receptors with adsorbed virus move together (patch) to pits coated with *clathrin* before moving into the cytosol to form small uncoated vesicles, which then fuse together as endosomes. A proton pump in the endosome lowers the pH to about 5. This acidic pH triggers the conformational change leading to the fusion of the viral and endosomal membranes, releasing the nucleocapsid into the cytosol. The endosomes combine with lysosomes, which eventually degrade any viral components contained within. This process is outlined in Figure 7.3. *Class II fusion proteins* are found in Flaviviruses (e.g. yellow fever virus, hepatitis C virus) and Togaviruses (e.g. rubella). They have different structural features from the class I fusion proteins, consisting of heterodimers. In the acidic endosome following receptor-mediated endocytosis, reorganization of the protein takes place with formation of active homotrimers and insertion of a hydrophobic fusion loop into the target membrane.

Viral entry via endocytosis can be independent of clathrin and dependent instead on *caveolae* or *lipid rafts*. These are areas of the membrane that

Table 7.2 Examples of viral receptors and co-receptors

Virus	Receptor
Coxsackievirus A21	CD55 (decay-accelerating protein), ICAM-1
Epstein–Barr virus	CD21 (complement receptor), HLA Class II, various integrins
Foot and mouth disease virus	Sialic acid, various integrins
Herpes simplex virus type 1	Heparan sulfate, herpesvirus entry-mediator A, nectin 1 and 2,various integrins
Human immunodeficiency virus type 1	CD4, chemokine receptors (CCR5, CXCR4)
Influenza virus A	Sialic acid
Lassa virus	β-dystroglycan
New World haemorrhagic fever arenaviruses	Transferrin receptor 1
Rabies virus	Nicotinic acetylcholine receptor, CD56 (neuronal cell adhesion molecule), low affinity nerve growth factor receptor
Respiratory syncytial virus	Heparan sulfate, ICAM-I
Rhinovirus	ICAM-I (majority of strains), low density lipoprotein receptor (minority of strains)
Rotavirus	Sialic acid, various integrins, heat shock protein 70
Severe acute respiratory syndrome coronavirus	Angiotensin-converting enzyme 2

CD, cluster of differentiation; HLA, human leukocyte antigen; ICAM, intercellular adhesion molecule.

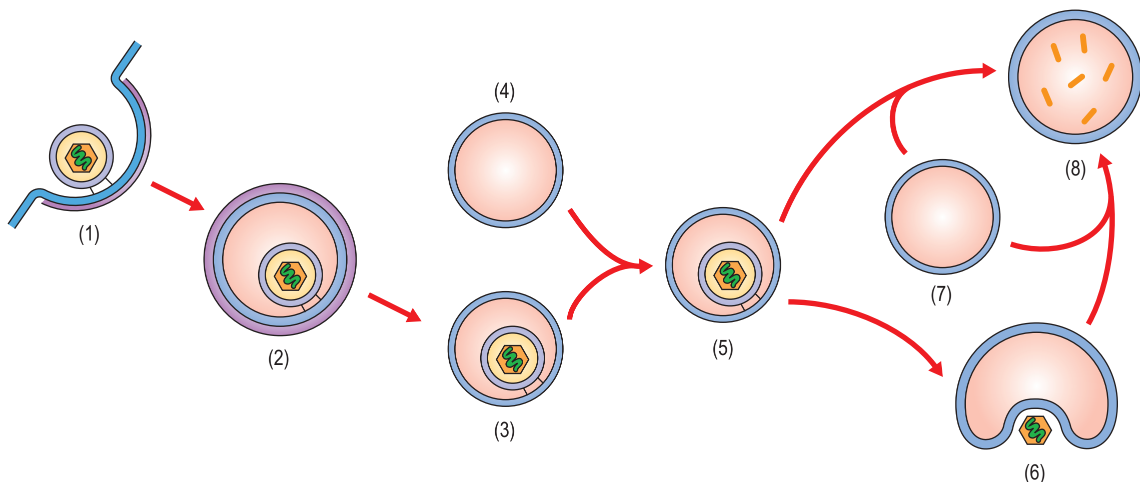

Fig. 7.3 Receptor-mediated endocytosis and entry of an enveloped virus. The virus attaches to specific receptors on the cell membrane (1) that patch at coated pits before being pinched off to form vesicles (2). These lose their coat (3) and fuse with other vesicles (4) to form endosomes (5). At the acid pH of endosomes, fusion of the viral envelope and the endosome membrane occurs, releasing the virus into the cytosol (6). (7) Fusion of the endosome with lysosomes leads to the final degradation of viral components and their return to the surface (8).

are rich in cholesterol and sphingolipids. Penetration into the cytosol occurs through the endoplasmic reticulum.

For many non-enveloped viruses, the mechanism by which they deliver their genomes across the host cell membrane in the absence of fusion is poorly understood. Recent findings indicate that such viruses may undergo programmed conformational changes following attachment, resulting in capsid disassembly and the release of small membrane-interacting peptides. These breach the membrane, thus allowing the viral genome to enter the cell.

Uncoating

Uncoating can take place at several stages and sites in the cell and, generally, is not a well understood process. Some viruses undergo conformational changes on attachment that result in the opening of the capsid and release of selected viral proteins and viral nucleic acid into the cell. For example, crystallography studies of picornaviruses have revealed that the myristic acid groups at one end of the VP4 structural protein interact with the host membrane; this causes VP4 and the viral genome to exit from the

capsid through a channel and to enter the cytosol. Enveloped viruses that enter by receptor-mediated endocytosis may be affected by the low pH in the endosome and the action of lysosomal enzymes. Uncoating can also take place in the cytosol or at the nuclear membrane. Reoviruses never fully uncoat, the viral genome remaining within a recognizable capsid structure. Poxviruses become uncoated in two stages. In the first, the outer layers and lateral bodies are removed within endosomal vesicles using host enzymes, and the core lies in the cytosol. Poxviruses carry their own DNA-dependent RNA polymerase, and this enzyme is used in the second stage to transcribe mRNA, which is translated into a special uncoating protein; this enables the final release of viral DNA from the core.

The final step in the complex uncoating process involves transport of the capsid (or the viral genome with, in some instances, viral enzymes and proteins) to the correct site in the cell to commence synthesis of the macromolecules that will comprise the new virions. Although details are not available for many viruses, it is clear that microtubules and microtubule-dependent motors are frequently involved in the transport. Some viruses stay in the cytosol for the remainder of their replication (e.g. poliovirus), but others proceed towards the nucleus where they are uncoated at the nuclear membrane before entry into the nucleus (e.g. herpesviruses) or enter the nucleus intact (e.g. papillomaviruses). Targeting to the nucleus depends on nuclear localization sequences found on the surface of the capsids. To gain access to the nucleus, the virus or its genome can either enter when the cell is undergoing mitosis (when the nuclear membrane is temporarily absent) or, more commonly, be delivered directly into the nucleoplasm though nuclear pore complexes.

Synthesis of viral components

The nucleic acid in viruses is either single or double stranded, circular or linear, in one piece or segmented. In addition, viruses vary enormously in their complexity, ranging from those with nucleic acid sufficient to code for only a few proteins, such as the papovaviruses, up to those coding for several hundred proteins, such as the poxviruses. Although every virus has a unique method of replicating and has a strict temporal control on the synthesis of components, each must present functional mRNA to the cell, so that new virally encoded polypeptides and nucleic acid can be synthesized using the normal cellular processes. Thus, only viruses that contain DNA and replicate in the nucleus can use solely cellular enzymes for transcription and translation. All other viruses must synthesize their mRNA by processes other than those found in uninfected cells. Seven different classes, six of which were first described by Baltimore in 1970, can be distinguished (Fig. 7.4). Conventionally in the scheme, nucleic acid of the same polarity or sense as mRNA is called 'positive' (+), and that of the opposite polarity or anti-sense is called 'negative' (-). Rather than be exhaustive, one or two illustrative examples from each class will now be described.

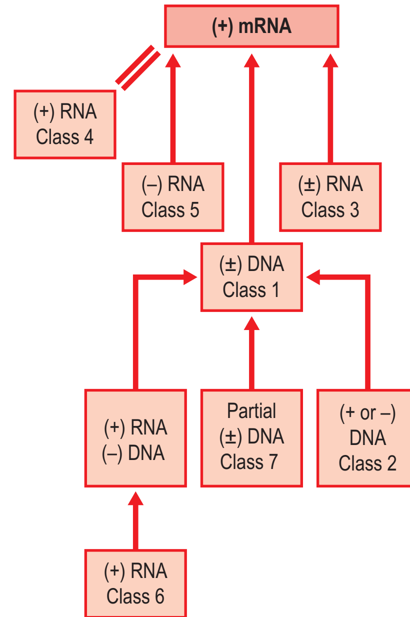

Fig. 7.4 Division of animal viruses into seven classes, based on mechanisms of transcription.

Class 1: Double-stranded DNA viruses

This comprises a very large group of viruses that contain double-stranded DNA in a linear form (e.g. herpesviruses, adenoviruses and poxviruses) or a circular form (e.g. papovaviruses). The poxviruses can be separated from the others, as their replication takes place entirely in the cytoplasm and they can code for all the factors required for their own transcription and genomic replication. In the remaining double-stranded DNA viruses, replication occurs in the nucleus and is dependent to some extent on host cell factors. Herpes simplex virus is used as an example (Fig. 7.5).

After uncoating at the nuclear pore, the viral nucleic acid enters the nucleus and, using the normal host cell mechanisms of transcription and translation, three groups of viral polypeptides are synthesized in a strict temporal fashion. They are called immediate early (α),

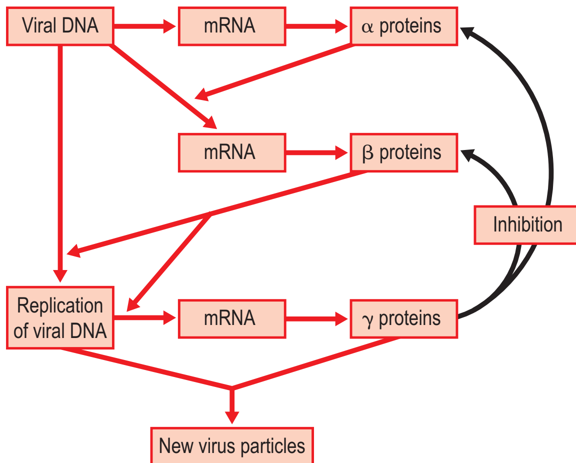

Fig. 7.5 Diagram of macromolecular synthesis during the replication of herpes simplex virus.

early (β) and late (γ). A component in the virus particle (α-transcription initiation factor [α-TIF], a γ protein), acting as a transactivator, induces the transcription of the first set of mRNAs. A second component of the viral tegument called virion host shut-off protein (VHS) inhibits host cell macromolecular synthesis, and all the metabolic energy of the cell is turned towards the production of new virus particles. The genes coding for the α, β and γ proteins have been mapped on the genome and, whereas the β and γ genes tend to be scattered, the α genes are located together. Among the early gene products are thymidine kinase and a virus-specific DNA polymerase. Most of the late proteins are structural proteins that inhibit the synthesis of the α and β proteins. Between β and γ protein synthesis, new viral DNA begins to be made, probably by circularization using a rolling circle model.

Class 2: Single-stranded DNA viruses

Parvoviruses comprise the sole family in this group. They are small, with DNA of about 5 kilobases. Some parvoviruses contain DNA of '–' polarity, and grow only in rapidly dividing cells; others contain either '+' or '–' DNA, and depend on coinfection with a helper virus for their replication. Parvoviruses use the cellular DNA polymerases to make the viral genome double stranded, called the replicative form. Priming is by the viral nucleic acid itself forming a loop at the 3′ terminus. This is followed by displacement of the parental DNA strand and synthesis of more DNA complementary to the template strand. Messenger RNAs are made using the appropriate DNA strand

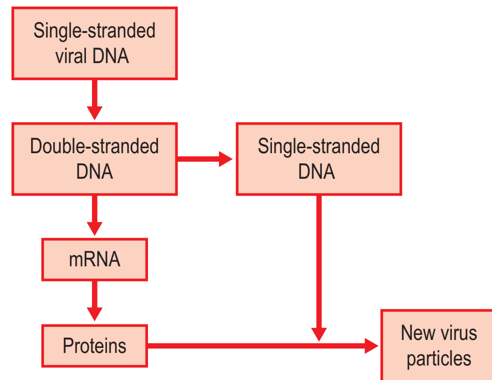

Fig. 7.6 Diagram of parvovirus replication.

as the template, and are translated into viral proteins (Fig. 7.6).

Class 3: Double-stranded RNA viruses

This group includes the reoviruses and rotaviruses. All members have segmented genomes and each RNA segment codes for a single polypeptide. Replication of viral nucleic acid, transcription and translation occur solely in the cytoplasm without nuclear involvement at any stage. Each infectious virus carries its own RNA-dependent RNA polymerase, an enzyme unique to some RNA viruses and not found in uninfected cells. It enables the transcription of one strand (-) into mRNAs, which are subsequently translated into viral proteins. The transcription is thus asymmetric and conservative: that is, only mRNAs are formed and the parental duplex is not broken apart. Each mRNA is later encapsidated and copied once to form double-stranded molecules (Fig. 7.7). Several hours pass between the '+' and '–' strands of the new virus particles being synthesized. Thus the replication of the double-stranded DNA and RNA viruses is very different.

Class 4: '+' single-stranded RNA viruses

This class comprises a large group of viruses containing RNA of the same polarity as mRNA. Because they code for all the proteins required during replication, the viral RNA extracted from the virions is infectious by itself. Poliovirus falls into this category, and is used as an example (Fig. 7.8). Macromolecular synthesis of viral components occurs entirely in the cytoplasm.

After entry of poliovirus into the cell, the viral RNA binds to ribosomes, acts as mRNA and is

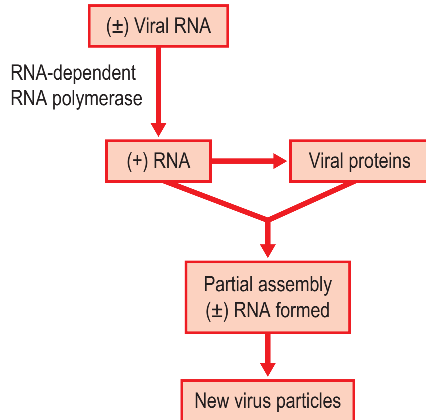

Fig. 7.7 Diagram of double-stranded RNA virus replication.

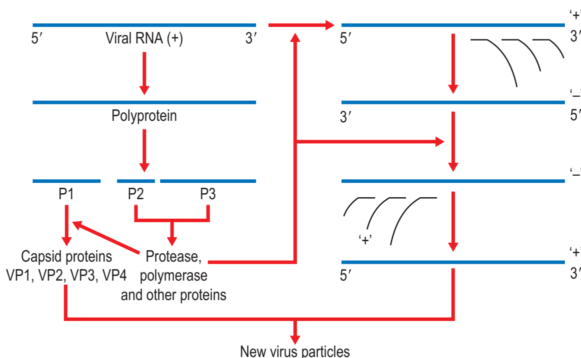

Fig. 7.8 Diagram of poliovirus replication.

translated in its entirety into one large polypeptide. This is then proteolytically cleaved to give the products RNA polymerase and protease enzymes and new capsid proteins. Using the polymerase enzyme, '–'-strand RNA is synthesized with the genomic RNA as the template, and a temporary double-stranded RNA is formed, called the replicative intermediate. The replicative intermediate consists of complete '+' RNA and numerous partially completed '–' strands. When the '–' strands are ready, they can be used as templates to make more '+'-strand RNA. This is required as genomic RNA for assembly into new virus particles and for transcription into more viral proteins.

At the same time as viral replication, host cell protein synthesis and RNA synthesis are inhibited. Initiation of translation of cellular mRNA requires the participation of a cap-binding protein at the 5′ end. Poliovirus induces the cleavage of this protein, and thus halts the synthesis of cellular proteins. The RNA genome of poliovirus does not have such a

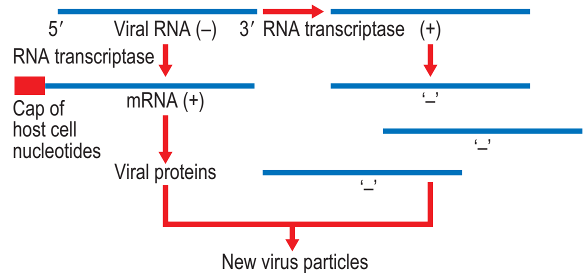

Fig. 7.9 Diagram of influenza virus replication.

cap although it has a small protein, called VPg, at the 5′ end. A special region near the 5′ end of the genome directs cap-independent initiation of protein synthesis.

Complex interactions between viral and cellular proteins are thought to determine how much viral RNA is used for new virus particles or is translated into protein. The capsid of poliovirus consists of 60 copies of each of four proteins, VP1, VP2, VP3 and VP4, forming the icosahedron. One of the first cleavages of the polyprotein produces VP1, which is then broken into VP0, VP3 and VP1. Finally, on assembly, VP0 is cleaved into VP4 and VP2, a process catalysed by VP0 itself.

Class 5: '–' single-stranded RNA viruses

Viruses of this group have single-stranded RNA of '–' polarity and must carry their own RNA transcriptase complex to be infectious, as the normal cellular enzymes are unable to replicate RNA. Influenza virus is an example (Fig. 7.9). It contains eight segments of '–'-strand RNA, plus the RNA transcriptase complex within each virus particle.

After entry into the cell by receptor-mediated endocytosis, transcription to viral mRNA occurs in the nucleus. Influenza virus is the only '–'-strand RNA virus to replicate in the nucleus. To initiate transcription, a nucleotide sequence of about 10–13 bases, found at the 5′ end of the cellular mRNAs and already capped, is used. This is cleaved from cellular mRNAs by an endonuclease activity of the viral RNA transcriptase complex. Thus, all the viral mRNAs have a 5′-terminal segment of the host cell mRNA.

Once the mRNAs have been generated, they are translated into polypeptides. Each genomic segment produces one mRNA, translated into one polypeptide except in two instances where, by RNA splicing of the original transcript, more than one mRNA is produced and therefore more than one protein. Unlike the transcription of mRNAs, the production of '+'-strand RNAs, required as intermediates to make the progeny

'–'-strand RNAs, proceeds without the need for primers.

There is much trafficking of viral polypeptides in the cell; the haemagglutinin, neuraminidase and M_2 protein are inserted in the plasma membrane, and the M_1 protein below this point on the membrane, whereas the nucleocapsid assembles around the viral RNAs in the nucleus.

Class 6: Retroviruses

Viruses of this group are unique as they contain single-stranded RNA (in the form of two identical subunits), yet they replicate via an integrated double-stranded DNA stage. Retroviruses are the only such family and the virus particles contain a reverse transcriptase complex, with RNA-dependent DNA polymerase activity, from which the name 'retrovirus' is derived. This enzyme is not found in uninfected cells.

After entry, synthesis of DNA complementary to the viral RNA occurs using the reverse transcriptase, originating at a primer binding site near the 5′ end of the viral genome. The primer is a specific transfer RNA (tRNA) and varies from one retrovirus to another (e.g. tRNAlys in HIV). Transcription proceeds towards the 5′ end, and is probably continued by a jump across to the 3′ end of the same molecule. In addition to RNA-dependent DNA polymerase activity, the reverse transcriptase complex has ribonuclease (RNAase) H activity, that is, it is able to digest RNA from a DNA–RNA hybrid (Fig. 7.10). The resulting single-stranded DNA is then made double stranded, using the reverse transcriptase as enzyme and starting from a purine-rich sequence. Thus, a linear double-stranded DNA form is produced, first found in the cytoplasm.

The viral RNA has a short sequence of about 12–235 bases repeated at each end. During replication there is generation of a longer repeat sequence, from 250–1000 nucleotides, at both ends of the DNA molecule. This is called the *long terminal repeat*; it contains the enhancer and promoter sequences controlling the expression of the viral genome as well as the sequence for the initiation of transcription. The linear double-stranded DNA is able to circularize, and is found in this form in the nucleus.

The next step is integration of the circular DNA into the host cell DNA. This is catalysed by an integrase carried by the virion. It is thought that the circular viral DNA is cleaved leaving staggered ends, and the cellular DNA similarly, to allow insertion of the viral DNA into the cellular DNA; the viral DNA is now called a *provirus*. The site of insertion is not thought to be specific. The provirus is co-linear with the original viral genome, and is always flanked by a 4–6-base pair direct repeat of the host DNA; this repeat is also found flanking transposons. The integrated state is a stable one and, as the DNA of the cell is replicated during cell growth, so the viral DNA is also replicated. Integration can result, on occasion, in cell transformation (see below).

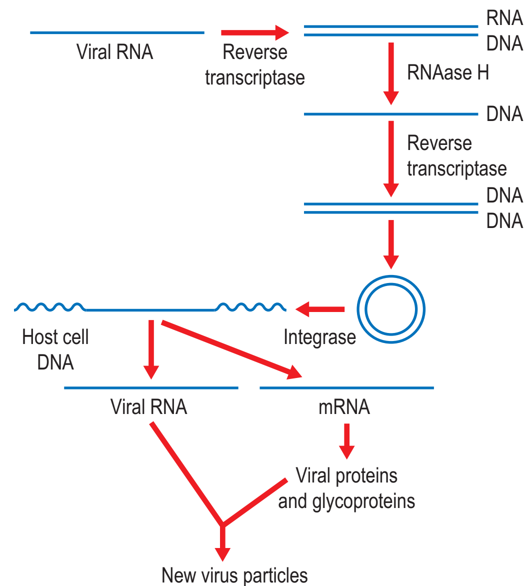

Fig. 7.10 Diagram of retrovirus replication.

The replication cycle is completed using the normal cellular RNA polymerase II to synthesize viral RNA and viral mRNAs, which are translated into polyproteins and processed into the final proteins found in the virus particle. A viral protease is responsible for many of these cleavages. The control of this stage is complex.

Class 7: Partial double-stranded DNA viruses

Hepadnaviruses are unique among the animal viruses in containing partial double-stranded DNA and replicating via an RNA intermediate, as shown in Figure 7.11. One example of this group is hepatitis B virus.

The first stage in the replication cycle is the production, in the nucleus, of fully double-stranded DNA, followed by the synthesis of single-stranded positive-sense RNA using the cellular DNA-dependent RNA polymerase. The RNA is transported into the cytoplasm and translated into the core protein, which encapsidates the RNA, together with newly synthesized viral RNA-dependent DNA polymerase (reverse

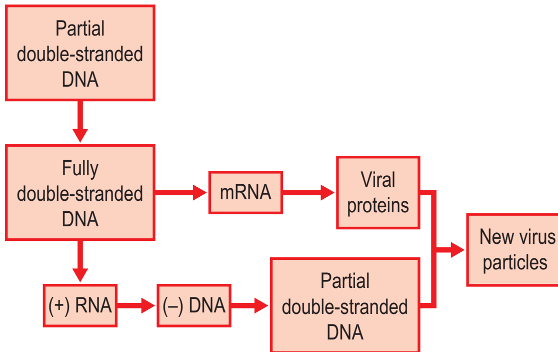

Fig. 7.11 Diagram of hepatitis B virus replication.

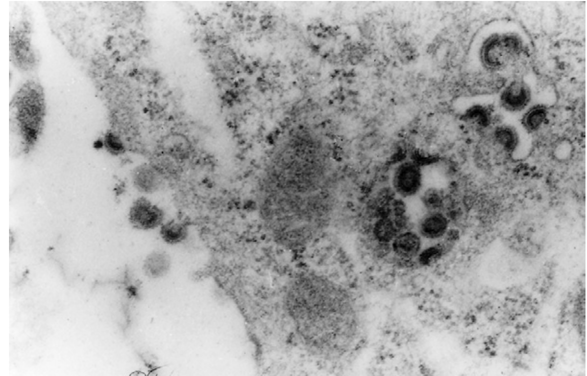

Fig. 7.12 Budding of retroviruses. ×70 000.

transcriptase). Then, using this enzyme, a complementary negative strand of DNA is made, while the RNA is degraded. The DNA is next transcribed into positive-sense DNA, and is found as partial double-stranded DNA in the new virus particles.

Assembly and release

After synthesis of viral proteins and viral nucleic acid, there is a stage of assembly called *morphogenesis*. Generally the components that will constitute the new virions are produced in high quantities, and the assembly process is probably rather inefficient. Assembly is followed by *release* of virus particles, the productive phase of the infection. The release is either through *cell lysis*, as used by many non-enveloped viruses, or through *budding*, in most cases without cell death, as used by many enveloped viruses. Recently some non-enveloped DNA viruses have been shown to code for a small basic regulatory protein (the agnoprotein) whose function is to act as a *viroporin*, resulting in membrane permeabilisation and the subsequent release of the newly synthesized virus particles. There is also evidence for active release without cell lysis for other non-enveloped viruses, perhaps via vesicles resembling autophagosomes that contain the newly synthesized viruses. In some cases, viruses can be transferred directly from the infected cell to the neighbouring cells, thus avoiding any exposure to the extracellular environment or to the immune response of the host. The transfer could be through tight junctions or at sites of synaptic contact.

Viruses that are released by killing the cell depend on the cell disintegrating to let them out. Here, morphogenesis may occur spontaneously once the capsid proteins have been made, the specificity depending on the amino acid sequence of the proteins. Thus the structural proteins of the viruses can form capsomeres by themselves, which then aggregate to form the procapsid, a structure without nucleic acid. Often there is proteolytic cleavage of a capsid protein to form the final virus particle, as has been described above for poliovirus. Details regarding the precise nature of the interaction between the nucleic acid and the structural proteins that make up the capsid are uncertain, despite extensive study. It is possible that the viral nucleic acid is inserted into the procapsid through a pore, or it might cause a structural reorientation of the procapsid, thereby becoming internalized. Alternatively, the capsomeres may accumulate around a condensed core of nucleic acid as the nucleic acid is being synthesized. Some evidence for specific viral components or cellular proteins, called scaffolding proteins, assisting the assembly process has been obtained.

The other major method of assembly and release is by budding. This can take place through the plasma membrane, thus releasing the virions from the cell (e.g. orthomyxoviruses and retroviruses; Fig. 7.12), or through internal membranes, such as the inner nuclear membrane in the case of the herpesviruses (Fig. 7.13B), followed by fusion of the vesicles containing the viruses with the plasma membrane. Envelope glycoproteins specified by the virus are synthesized by essentially the same mechanism as cellular membrane glycoproteins. The viral proteins destined to become envelope proteins contain a sequence of 15–30 hydrophobic amino acids, known as the signal sequence. This sequence binds the growing polypeptide chain to a receptor on the cytoplasmic side of the rough endoplasmic reticulum and enables its passage through the membrane. Glycosylation occurs in the lumen of the rough endoplasmic reticulum, and the proteins are transported to the Golgi apparatus. There they are further glycosylated and acylated before transport to the plasma membrane, the direction probably

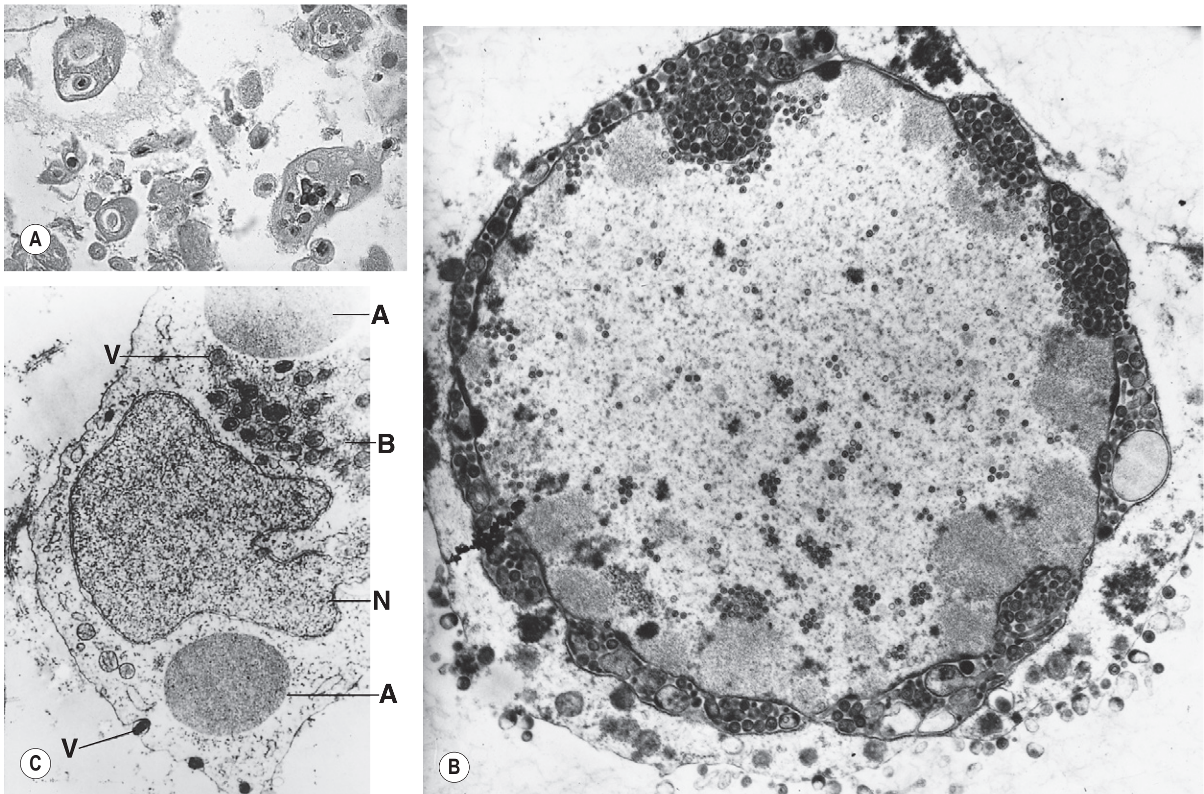

Fig. 7.13 Effects of viruses on cells. (A) Light microscopy of a skin lesion due to herpesvirus infection, showing cell fusion and intranuclear inclusions (Cowdry type A). ×60. (B) Electron micrograph of a cell infected with herpes simplex virus. Assembly of capsids within nucleus and enveloped virus between layers of nuclear membrane. ×9000. (C) Type A (accumulation of viral protein) and type B (virus factory) inclusions (identified as A and B, respectively) in the cytoplasm of a poxvirus-infected cell. V, virus; N, the cell nucleus. ×700.

determined by a sorting signal in the polypeptide sequence. In some cases, the signal directs the viral glycoprotein to one surface of the cell only; for example, orthomyxoviruses bud only from the outer (apical) surface of epithelial cells, whereas rhabdoviruses bud only from the inner (basal) surface. The viral glycoproteins are very important in terms of antigenicity as the hydrophilic domains protrude from the surface of the cell, with the N terminus being furthest away, and change the surface structure significantly. They remain anchored in the membrane via a hydrophobic domain near the carboxyl (C) terminus. After insertion into the membrane, the viral glycoproteins accumulate together to form oligomers; at the same time the host cell glycoproteins move away. At the C terminus of the viral glycoproteins, there is frequently a short hydrophilic sequence that remains inside the cell and is assumed to interact with the internal components of the virus during assembly. Lipid rafts function as microdomains for the accumulation of many viral glycoproteins, and may also initiate the actual budding sequence. It is not known how the nucleocapsids are directed to the assembly site. Once there, they are engulfed by the membrane; this process requires bending of the membrane, leading to its outward curvature. In the case of the class I fusion proteins described above, a final cleavage of the glycoprotein is required to make the virus infectious. For example, the haemagglutinin H_0 of influenza virus is cleaved into two peptides, HA_1 and HA_2, linked by disulphide bridges. The bud is completed by the fusion of the two apposing membranes, and it finally separates from the plasma membrane of the host to become a new infectious virus. For retroviruses, budding occurs by highjacking a cellular pathway that normally creates vesicles that bud into late endosomal compartments called multivesicular bodies. These viruses then exit through the plasma membrane or via exosomes. During or shortly after budding, the viral protease enzyme cleaves at specific sites within precursor proteins to produce the proteins found in the mature virus particles.

Several thousand virus particles can be produced per infected cell, although this number varies considerably with virus type and host cell type. The budding viruses tend to be released slowly over several hours, whereas the lytic ones are released together. Only a few of the newly formed virus particles are infectious, as indicated by a high ratio of particles to infectious virions. Presumably most do not have the correct complement of proteins, enzymes or viral nucleic acid, or have been assembled incorrectly.

Evidence has accumulated to indicate that, for some viruses at least, perturbation of the normal cell metabolism during replication can stimulate cell death by *apoptosis*. Several virus-specific factors have been identified as inducers of apoptosis, such as by causing DNA strand breaks in the case of a parvovirus, by stabilization of the tumour suppressor gene product p53 in the case of Epstein–Barr virus, or by receptor signalling in the case of HIV. The advantage to the virus could be that the spread of the infection is enhanced as the entire cellular contents, including progeny viruses, are packaged into membrane-bound apoptotic bodies that are then taken up by adjacent cells. In contrast, other viral proteins have been revealed that block or delay apoptosis, presumably until sufficient progeny viruses have been produced within the cells. In this case, the factors target specific stages of the apoptotic pathway. For example, caspases are inhibited during poxvirus infections, the action of interferon is downregulated during influenza virus infections and p53 is destroyed during some human papillomavirus infections. Therefore the susceptibility of the host cell to apoptosis depends on the acute death pathways in the cell itself and the range of apoptotic modulators induced by the infecting viruses.

Microscopy of infected cells

It is possible to observe effects on the host cell microscopically. Firstly, there may be morphological changes called *inclusion bodies* in the infected cell, seen by altered staining characteristics. The inclusion bodies are nuclear or cytoplasmic and vary in their composition. They can consist of viral factories in which morphogenesis occurs, crystalline arrays of virus particles ready for release, overproduction of a particular viral protein or proteins, or some aberrant cellular structure, such as clumped chromatin. Virally encoded non-structural proteins are likely to be involved in forming the matrix of these structures and in recruiting viral components to them. Some examples of inclusion bodies are shown in Figure 7.13. Secondly, the cells may be killed by the viral infection. There are several possible reasons for this, including factors produced by the virus that induce apoptosis (see above). It is likely that the accumulation of viral structural proteins is toxic for the cells in some cases. In addition, some viruses, such as herpes simplex virus and the poxviruses, inhibit host cell macromolecular synthesis from an early stage in the replication cycle, leading to structural and functional damage. Plasma membrane function and permeability change, and lysosomal membranes begin to break down, allowing leakage of the contents with degradative activity into the cytoplasm. There may also be marked effects on the cytoskeleton. These changes lead to a *cytopathic effect*, seen clearly in cell culture. It can take several forms, one of the most common being *cell rounding* and subsequent detachment from the solid surface (Fig. 7.14B). Another is the formation of a *syncytium,* whereby the membranes of adjacent infected cells fuse and a giant cell is formed containing many nuclei (Fig. 7.13A and 7.14C). In some cases, the nuclei fuse to make hybrid cells, a property that has been exploited in monoclonal antibody production.

NON-CYTOCIDAL PRODUCTIVE INFECTIONS

Some viruses are able to infect cells productively but the cells are not killed by the replication process.

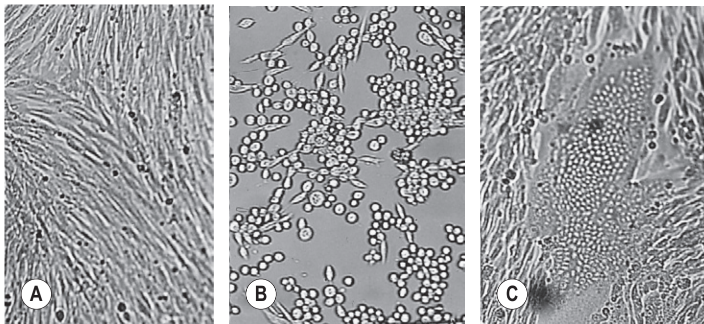

Fig. 7.14 Cytopathic effects: (A) uninfected fibroblast cells; (B) Cell rounding due to herpes simplex virus; (C) Syncytium formation or cell fusion due to respiratory syncytial virus. All unstained. ×65.

Viruses that are released by budding frequently come into this category. The cell type used for the infection is critical and, presumably, any inhibitory effect of the virus on the cellular metabolism does not take place. This type of interaction may lead to a *persistent infection* in which infected cells and viruses coexist over a long period of time. There will, however, be antigenic changes in the infected cells, often the insertion of viral glycoproteins in the plasma membrane. This can be exploited for the detection of a virus; for example, when influenza virus is cultured in monkey kidney cells, virus is produced from the cells but there is no immediate cytopathic effect. However, there is insertion of viral haemagglutinin into the plasma membrane during replication. Thus, when red blood cells of certain species are added to the infected cells, they adhere to the haemagglutinin and can be seen microscopically. This phenomenon is called *haemadsorption*.

ABORTIVE (NON-PRODUCTIVE) INFECTIONS

Some viruses are unable to infect cells because they cannot adsorb to them; the cells are therefore called *resistant*. In other cases viruses are able to infect cells but are not produced from them; such infection is called *non-productive* or *abortive*, and the cells are *non-permissive*. Often there is a block at one stage of the replication cycle owing to the absence of a cellular function essential for viral replication. This can also occur in a permissive cell if the viral genome itself is *defective* in some way, so that the replication cannot be completed. This can happen in two ways:

1. The virus is too small to code for all the proteins required for replication. An example is provided by the adeno-associated viruses belonging to the Parvoviridae family. They are unable to replicate on their own but depend on a second 'helper' virus infecting the same cell and providing the essential function that they lack. Adenoviruses act as the helper viruses and are thought to activate transcription of the parvovirus genome in the infected cell.
2. Abortive infections can arise as a result of viral mutation. In fact, only a few amino acid substitutions in selected proteins of the virus can change the nature of a lytic infection. In some cases the non-productive infection can be converted into a persistent one, in which new virus particles are synthesized; three examples are given below.

Temperature-sensitive mutants

Here the wild-type virus has mutated to produce a variant – a temperature-sensitive mutant – that lacks an essential gene function at the *non-permissive temperature*, normally above 38°C. This is thought to be due to the thermal instability of the secondary or tertiary structure of a particular protein. The temperature can be lowered, generally to below 35°C, to a level called permissive, where the defect is no longer functional and the infection becomes productive. However, temperature-sensitive mutants tend to be less cytopathic than the wild type, even at permissive temperatures, probably because they synthesize less mRNA and protein. Thus, within a cell culture, a persistent infection can result with a balance between infected and uninfected cells together with virus production. Temperature-sensitive mutants have been used to identify the gene responsible for a particular replication step and to map where functions lie on the viral genome. It is possible to place these mutants into complementation groups where, at the non-permissive temperature, the defect in one temperature-sensitive mutant is compensated for by a second one with a defect in a different gene.

Defective interfering particles

It has been known for many years that, when cells are infected at high multiplicity, there will be a number of virus particles among the progeny with genomes shorter than normal, containing at least one deletion – so-called *defective interfering particles*. These particles cannot replicate themselves, although they are able to infect new cells. However, they can replicate in the presence of helper virus, often the parental virus, which compensates for the lack of a particular gene or gene cluster. The defective particles retain an origin of replication and the ability to form capsids. One of their important properties is that they *interfere* with the replication of normal parental viruses because, first, less time and energy are required to replicate the defective genome compared with the full-length genome and, second, the transcriptase complex has a greater affinity for the defective genome than the full-length one. Hence defective interfering particles, as their numbers increase, have a greater and greater effect on the replication of parental virus. It is possible to obtain an in vitro cell culture in which infected and uninfected cells together with infectious virus and defective particles are in balance for a prolonged period of time. Thus a steady state exists and the infection is persistent.

Abortive infections maintained by interferons

The final example of abortive infections arises from the action of *interferons* in infected cell cultures. Interferons are produced from virally infected cells and can protect other cells from attack by viruses. These molecules, of α or β type, inhibit various stages of the viral replication cycle, especially polypeptide synthesis (see Ch. 10 for details). In cell culture, persistent infections can be obtained when the antiviral effects of interferons protect sufficient cells from the cytolytic effects of viral replication to allow cells and viruses to coexist.

It should be noted that some viruses can mutate so that they replicate poorly, if at all, in some cell types in vitro and in vivo, or they replicate normally but are less virulent in vivo. These mutated strains are described as *attenuated* and are the basis of most live viral vaccines. For example, the three serotypes used in the Sabin poliovirus vaccine have attenuating mutations in the non-coding regions of the viral RNA, leading to a failure to replicate in the brain and less efficient replication than the wild-type strains at the primary site of infection in the gut. Attenuation can be achieved by culturing the viruses repeatedly in cells other than those of the normal host or by culturing at non-physiological temperatures.

LATENCY

Latency represents a type of persistence whereby the virus is present in the form of its genome only and there is limited expression of viral genes. The genome is found either integrated into the host cell chromosome or as a circular non-integrated episome. It is maintained throughout cell division when the host cell replicates. Latent infections are more common with DNA viruses than RNA viruses, perhaps because no mechanisms exist to maintain RNA for long periods of time intracellularly. One example of latency is provided by Epstein–Barr virus, which persists in B lymphocytes as episomal viral DNA with limited transcription of viral genes, probably around 11 protein products being expressed. These ensure maintenance of the viral genome in dividing cells, prevent apoptosis of the host cells, and help to evade immune responses. For herpes simplex virus, latency occurs in neuronal cells with the viral genome being maintained in the nucleus as an episome. All the lytic genes are switched off but one set of transcripts, the latency-associated transcripts (LATs), is abundantly expressed. The LATs can inhibit apoptosis and thus contribute to persistence of the virus. However, whether viral gene expression is substantially prevented by the action of the LATs or by the immune response of the host, such as via the local production of interferons and cytokines, is uncertain. Specific stimuli can trigger the reactivation of the virus from the latent state, and the infection becomes productive with the appearance of new virions.

TRANSFORMATION

In this type of virus–cell interaction, the virus infects the cell non-productively and is found in the form of viral DNA, either integrated in the host cell DNA or unintegrated, or in both states. The properties of the cells are changed dramatically, a process called *transformation*. Transformed cells have similar properties to tumour cells, and a detailed study of the mechanism of viral transformation has led to increased understanding of the molecular basis of cancer. Only members of some virus families are able to transform cells. These include herpesviruses, adenoviruses, hepadnaviruses, papovaviruses and poxviruses of the DNA viruses, and, of the RNA viruses, only retroviruses. The type of cell infected and the species are also important. It should be noted that transformation is a rare event: at most only 1 in 10^5 cells infected by a particular virus will become transformed.

Some of the main properties of transformed cells that distinguish them from normal cells are listed below:

- loss of contact inhibition of growth
- can grow to high saturation density
- less requirement for serum factors
- indefinite number of cell divisions
- expression of viral antigens
- absence of fibronectin
- fetal antigens often present
- changes in agglutinability by plant lectins
- induction of tumours in experimental animals.

One of the most striking changes is the loss of contact inhibition of growth, whereby cells that normally grow in an ordered fashion beside their neighbours and stop dividing when they touch one another now grow on top of each other and lose their orientation with respect to each other (Fig. 7.15). As a result they reach much higher densities. They have less requirement for serum factors in the medium and can be cultured in suspension without being attached to a solid surface (anchorage independent). Normal cells have a limited number of cell divisions, called the *Hayflick limit*, that they can undergo in vitro before

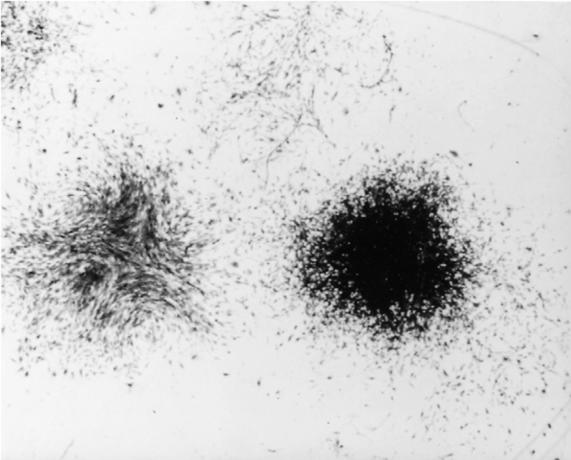

Fig. 7.15 Colonies of human embryo fibroblasts growing normally (left) and following viral transformation (right).

apoptosis; for example, the Hayflick limit for cells taken from a human fetus is around 60 divisions. Transformed cells no longer have this limit and thus can grow and divide indefinitely. There are many changes in the surface properties of transformed cells. Often viral-specific antigens are found, particularly ones synthesized early in the replication cycle. Fibronectin, a surface glycoprotein thought to be important in keeping cells together in a tissue or organ, is no longer found. Commonly fetal antigens are expressed, and the agglutinability of cells by plant lectins changes, demonstrating alterations in the distribution of membrane glycoproteins. Finally, some transformed cells form tumours when injected into susceptible animals. Often these animals have to be immunocompromised in some way before tumours are produced, or the cells inserted into an immunologically protected site, such as the cheek pouch of the hamster. In addition, it is important to appreciate that by no means all transformed cells will form tumours. It is thought that there are degrees of transformation and that several stages have to be completed before the cells are fully transformed and equivalent to malignant tumour cells. Viral transformation may represent only the first step or a single step in such a pathway. There are no in vitro markers that determine the degree of transformation; thus the potential ability of the transformed cell to produce tumours in experimental animals cannot be predicted, as yet.

All of the viruses that cause transformation in vitro have a similar interaction with the host cell (Fig. 7.16). The initial stages are exactly as described above for the productive infections. There is attachment, entry, uncoating and, in most but not all cases, selected viral genes are expressed as proteins, giving the cell new antigenic properties. At this stage the viral nucleic acid becomes integrated in the host cell DNA, probably not at a specific site, or it circularizes and is maintained in a non-integrated episomal form in the nucleus. The association is a stable one, so that when the host cell DNA is replicated, the viral nucleic acid is also replicated and the number of viral genome copies per cell remains constant over many cell generations. Thus, transformation is a heritable alteration. With some viruses, such as Epstein–Barr virus and the papovaviruses, the whole viral genome is normally integrated, whereas with others, such as herpes simplex virus and adenoviruses, only part of the viral genome is integrated and the remainder is lost.

Recent work in this area has concentrated on the molecular events surrounding transformation and in analysing the functions of the viral proteins found in transformed cells. Two examples of transforming viruses, one RNA and the other DNA, are described briefly below to illustrate the approaches taken. Both are associated with human tumours.

The first is human T lymphotropic or T cell leukaemia virus type I (HTLV-I), a retrovirus, which is found in $CD4^+$ T cells of patients with adult T cell leukaemia. It is able to transform $CD4^+$ lymphocytes in vitro with integration of the DNA provirus. Genetic analysis has revealed that the viral genome can code for several non-structural proteins, including one of special interest called Tax, an oncoprotein of molecular weight 40 kDa. This protein, which has no cellular homologue, is able to activate transcription in the long terminal repeat of the integrated virus. Tax also affects the transcription of a remarkable number of cellular genes that are involved in cell cycle control and the cellular response to DNA damage. In short, it functions in a complex manner to promote cell proliferation, to accumulate DNA damage with the loss of genomic integrity, and to inhibit apoptosis. However, it is unlikely that Tax expression alone leads to the end-point of leukaemia and further, so far unexplained, molecular events occurring over a period of several years, are probably necessary.

The second example is human papillomavirus type 16 (HPV-16), found as integrated DNA in many cases of carcinoma of the cervix. In vitro this virus is able to transform most types of human epithelial cells, including keratinocytes. The viral proteins responsible for transformation are the products of two genes, *E6* and *E7*, which are found in cervical tumour cells. The interaction between E6 and E7 oncoproteins and their cellular targets is required to maintain the malignant phenotype. Both proteins have multiple functions but

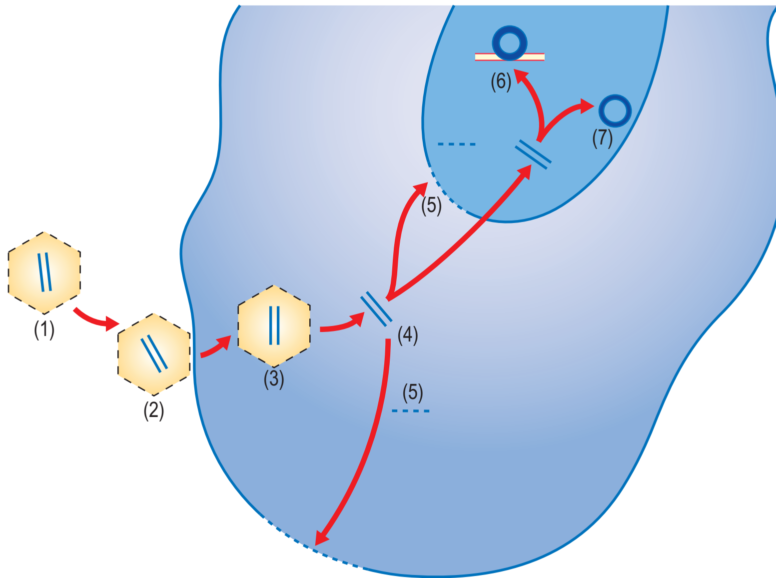

Fig. 7.16 Simplified diagram showing the events of viral transformation. The hypothetical virus (1) attaches (2), enters (3), is uncoated (4), and usually some early viral proteins are synthesized (5) followed by integration of the viral genome in the host cell DNA (provirus) (6) or formation of a circular non-integrated genomic DNA (episome) (7).

it is probably most important that E6 interacts with p53, and E7 with retinoblastoma protein, thereby inactivating them. As both p53 and retinoblastoma protein act as cellular growth-suppressing proteins, loss of their functions is likely to lead to transformation. In addition, integration of the viral genome normally involves the disruption of the *E2* gene, the product of which is required to stop transcription of the E6 and E7 promoters, and therefore the continued expression of the E6 and E7 proteins results. Further properties of the E6 and E7 proteins include the inhibition of apoptosis, overriding of cell cycle controls, chromosome destabilisation and, in vivo, various mechanisms to evade local immune responses.

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