Chapter 1

Towards a definition of a virus

Viruses occur universally, but they can only be detected indirectly. Viruses are obligate intracellular parasites that require a host within which they replicate. Although they are well known for causing disease, most viruses coexist peacefully with their hosts.

Chapter 1 Outline

Viruses are arguably the most ubiquitous and widespread group of organisms on the planet, with every animal, plant and protist species susceptible to infection. The efficiency of replication demonstrated by viruses is such that the infection of a single host can generate more new viruses than there are individuals in the host population. For example, a single human infected with influenza virus can shed sufficient virus particles to be theoretically capable of infecting the entire human population. While not every species has been examined for the presence of viruses, those that have been tested have all yielded up new virus isolates. Further, not only do viruses occur universally but each species has its own specific range of viruses that, by and large, infects only that species. In recent years, the application of new nucleic acid sequencing techniques has demonstrated that a vast array of previously unknown viruses remains to be studied.

Current estimates of the number of individual viruses on earth suggest that they considerably exceed the total number of stars in the known universe, i.e. more than 10^{23} (100 sextillion). This vast number raises questions as to what the viruses are doing there, and what selective advantage, if any, they afford to the species that host them. The answer to the first of these is the same as if the question was posed about any organism – it is simply occupying a particular environmental niche which, in the case of a virus, is another species. The answer to whether or not any benefit accrues for hosting a virus is usually not known, though the adverse effects of virus infections are all too well known. However, it is clear that, despite their adverse effects and the dramatic depictions of viruses in popular media and cinema, viruses have not made their hosts extinct.

Introduction to Modern Virology, Seventh Edition. N. J. Dimmock, A. J. Easton and K. N. Leppard.

1.1 Discovery of viruses

Although much is known about viruses (Box 1.1), it is instructive and interesting to consider how this knowledge came about. It was only just over 100 years ago, at the end of the 19th century, that the germ theory of disease was formulated, and pathologists were then confident that a causative micro-organism would be found for each infectious disease. Further, they believed that these agents of disease could be seen with the aid of a microscope, could be cultivated on a nutrient medium, and could be retained by filters. There were, admittedly, a few organisms which were so fastidious that they could not be cultivated in the laboratory but the other two criteria were satisfied. However, in 1892, Dmitri Iwanowski was able to show that the causal agent of a mosaic disease of tobacco plants, manifesting as a discoloration of the leaf, passed through a bacteria-proof filter, and could not be seen or cultivated. Iwanowski was unimpressed by his discovery, but Beijerinck repeated the experiments in 1898, and became convinced that this represented a new form of infectious agent which he termed *contagium vivum fluidum*, what we now know as a virus. In the same year, Loeffler and Frosch came to the same conclusion regarding the cause of foot-and-mouth disease. Furthermore, because foot-and-mouth disease could be passed from animal to animal, with great dilution at each passage, the causative agent had to be reproducing and thus could not be a bacterial toxin. Viruses of other animals were soon discovered. Ellerman and Bang reported the cell-free transmission of chicken leukaemia in 1908, and in 1911 Rous discovered that solid tumours of chickens could be transmitted by cell-free filtrates. These were the first indications that some viruses can cause cancer (see Chapter 25).

Finally, bacterial viruses were discovered. In 1915, Twort published an account of a glassy transformation of micrococci. He had been trying to culture the smallpox agent on agar plates but the only growth obtained was that of some contaminating micrococci. Following prolonged incubation, some of the colonies took on a glassy appearance, and once this occurred no bacteria could be subcultured from the affected colonies. If some of the glassy material was added to normal colonies, they too took on a similar appearance, even if the glassy material was first passed through very fine filters to

Box 1.1

Properties common to all viruses

- Viruses have a nucleic acid genome of either DNA or RNA.
- Compared with a cell genome, viral genomes are small, but genomes of different viruses range in size by over 100-fold (ca 3000 nt to 1,200,000 bp)
- Small genomes make small particles – again with a 100-fold size range.
- Viral genomes are associated with protein that at its simplest forms the virus particle, but in some viruses this nucleoprotein is surrounded by further protein or a lipid bilayer.
- The outermost proteins of the virus particle allow the virus to recognise the correct host cell and gain entry.
- Viruses can only reproduce in living cells: they are obligate parasites.

exclude all but the smallest material. Among the suggestions that Twort put forward to explain the phenomenon were either the existence of a bacterial virus or the secretion by the bacteria of an enzyme which could lyse the producing cells. This idea of self-destruction by secreted enzymes was to prove a controversial topic over the next decade. In 1917, d'Hérelle observed a similar phenomenon in dysentery bacilli. He observed clear spots on lawns of such cells, and resolved to find an explanation for them. Upon noting the lysis of broth cultures of pure dysentery bacilli by filtered emulsions of faeces, he immediately realized he was dealing with a bacterial virus. Since this virus was incapable of multiplying except at the expense of living bacteria, he called his virus a *bacteriophage* (literally a bacterium eater), or *phage* for short.

Thus the first definition of these new agents, the viruses, was presented entirely in negative terms: they could not be seen, could not be cultivated in the absence of cells and, most important of all, were not retained by bacteria-proof filters. However, these features define key characteristics of viruses: they are small parasites that require a host in which they replicate.

1.2 Multiplication of viruses

Early studies focused on establishing the nature of viruses. d'Hérelle believed that the infecting phage particle multiplied within the bacterium and that its progeny were liberated upon lysis of the host cell, whereas others believed that phage-induced dissolution of bacterial cultures was merely the consequence of a stimulation of lytic enzymes endogenous to the bacteria. Yet another school of thought was that phages could pass freely in and out of bacterial cells and that lysis of bacteria was a secondary phenomenon not necessarily concerned with the growth of a phage. It was Delbruck who ended the controversy by pointing out that two phenomena were involved: lysis from within and lysis from without. The type of lysis observed was dependent on the ratio of infecting phages to bacteria (referred to as the *multiplicity of infection*). At a low multiplicity of infection (with the ratio of phages to bacteria no greater than 2:1), then the phages infect the cells, multiply and lyse the cells from within. When the multiplicity of infection is high, i.e. many hundreds of phages per bacterium, the cells are lysed directly, and rather than an increase in phage titre there is a decrease. Lysis is due to weakening of the cell wall when large numbers of phages are attached.

Convincing support for d'Hérelle's hypothesis was provided by the one-step growth experiment of Ellis and Delbruck (1939). A phage preparation such as bacteriophage λ (lambda) is mixed with a suspension of the bacterium *Escherichia coli* at a multiplicity of infection of 10 infectious phage particles per cell, ensuring that virtually all cells are infected. Then, after allowing 5 minutes for the phage to attach, the culture is centrifuged to pellet the cells and attached phage. Medium containing unattached phage is discarded. The cells are then resuspended in fresh medium. Samples of the culture are withdrawn at regular intervals, cells and medium are separated and assayed for infectious phage. The results obtained are shown in Fig. 1.1. After a latent period of 17 minutes during which no phage increase is detected in cell-free medium, there is a sudden rise in the detection of infectious phage in the medium. This 'burst' size represents the average of many different bursts from individual cells, and can be calculated from the total virus yield/number of cell infected. The entire growth cycle here takes around 30 minutes, although this will vary with different viruses and cells. The amount of cell-associated virus is determined by taking the cells pelleted from the medium, disrupting

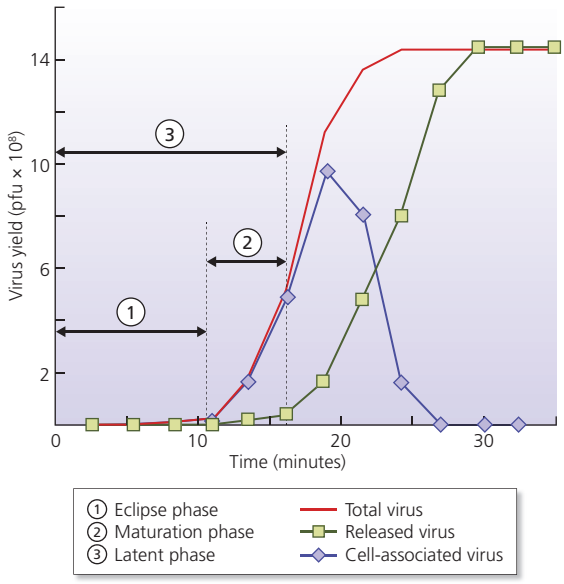

Fig. 1.1 A one-step growth curve of bacteriophage λ following infection of susceptible bacteria (*Escherichia coli*). During the *eclipse phase*, the infectivity of the cell-associated, infecting virus is lost as it uncoats; during the *maturation phase* infectious virus is assembled inside cells (cell-associated virus), but not yet released; and the *latent phase* 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. A consideration of the methods used to determine the yield of viruses is given in Chapter 5.

them and assaying for virus infectivity as before. The fact that virus appears inside the cells before it appears in the medium demonstrates the intracellular nature of phage replication. It can be seen also that the kinetics of appearance of intracellular phage particles are *linear*, not exponential. This is consistent with particles being produced by assembly from component parts rather than by binary fission.

1.3 The virus multiplication cycle

We now know a great deal about the processes which occur during the multiplication of viruses within single cells. The precise details vary for individual viruses but have in common a series of events marking specific phases in the multiplication cycle. These phases are summarized in Fig. 1.2 and are considered in detail in Part II of this book. The first stage is that of **attachment** when the virus attaches to the potential host cell. The interaction is specific, with the virus attachment protein(s) binding to target receptor molecules on the surface of the cell. The initial contact between a virus and host cell is a dynamic, reversible one often involving weak electrostatic interactions. However, the contacts quickly become much stronger with more stable interactions which in some cases are essentially irreversible. The attachment phase determines the specificity of the virus for a particular type of cell or host species. Having attached to the surface of the cell, the virus must effect entry to be able to replicate in a process called **penetration** or **entry**. Once inside the cell, the genome of the virus must become available. This is achieved by the loss of many, or all, of the proteins that make up the particle in a process referred to as **uncoating**. For some viruses, the entry and uncoating phases are combined in a single process. Typically, these first three phases do not require the expenditure of energy in the form of ATP hydrolysis. Having made the virus genome available it is now used in the **biosynthesis** phase when genome replication, transcription of mRNA and translation of the mRNA into protein occur. The process of translation uses ribosomes provided by the host cell and it is this requirement for the translation machinery, as well as the need for molecules for biosynthesis, that makes viruses obligate intracellular parasites. The newly-synthesized

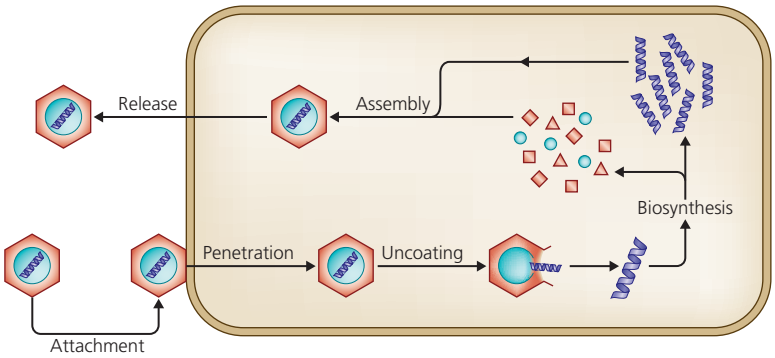

Fig. 1.2 A diagrammatic representation of the six phases common to all virus multiplication cycles. See text for details.

genomes may then be used as templates for further rounds of replication and as templates for transcription of more virus mRNA in an amplification process which increases the yield of virus from the infected cells. When the new genomes are produced, they come together with the newly-synthesized virus proteins to form progeny virus particles in a process called **assembly**. Finally, the particles must leave the cell in a **release** phase after which they seek out new potential host cells to begin the process again. The particles produced within the cell may require further processing to become infectious and this **maturation** phase may occur before or after release.

Combining the consideration of the steps which make up a virus multiplication cycle with the information in the graph of the results of a single step growth curve, it can be seen that during the eclipse phase the virus is undergoing the processes of attachment, entry, uncoating and biosynthesis. At this time, the cells contain all of the elements necessary to produce viruses but the original infecting virus has been dismantled and no new infectious particles have yet been produced. It is only after the assembly step that we see virus particles inside the cell before they are released and appear in the medium.

1.4 Viruses can be defined in chemical terms

The first virus was purified in 1933 by Schlessinger using differential centrifugation. Chemical analysis of the purified bacteriophage showed that it consisted of approximately equal proportions of protein and deoxyribonucleic acid (DNA). A few years later, in 1935, Stanley isolated tobacco mosaic virus in paracrystalline form, and this crystallization of a biological material thought to be alive raised many philosophical questions about the nature of life. In 1937, Bawden and Pirie extensively purified tobacco mosaic virus and showed it to be nucleoprotein containing ribonucleic acid (RNA). Thus virus particles may contain either DNA or RNA. However, at this time it was not known that nucleic acid constituted genetic material.

The importance of viral nucleic acid

In 1949, Markham and Smith found that preparations of turnip yellow mosaic virus comprised two types of identically sized spherical particles, only one of which contained

nucleic acid. Significantly, only the particles containing nucleic acid were infectious. A few years later, in 1952, Hershey and Chase demonstrated the independent functions of viral protein and nucleic acid using the head-tail virus, bacteriophage T2 (Box 1.2).

In another classic experiment, Fraenkel-Conrat and Singer (1957) were able to confirm by a different means the hereditary role of viral RNA. Their experiment was based on the earlier discovery that particles of tobacco mosaic virus can be dissociated into their protein and RNA components, and then reassembled to give

Box 1.2

Evidence that DNA is the genetic material of bacteriophage T2. The Hershey-Chase experiment

Bacteriophage T2 was grown in *E. coli* in the presence of ^{35}S (as sulphate) to label the protein moiety, or ^{32}P (as phosphate) to mainly label the nucleic acid. Purified, labelled phages were allowed to attach to sensitive host cells and then given time for the infection to commence. The phages, still on the outside of the cell, were then subjected to the shearing forces of a Waring blender. Such treatment removes any phage components attached to the outside of the cell but does not affect cell viability. Moreover, the cells are still able to produce infectious progeny virus. When the cells were separated from the medium, it was observed that 75% of the ^{35}S (i.e. phage protein) had been removed from the cells by blending but only 15% of the ^{32}P (i.e. phage nucleic acid) had been removed. Thus, after infection, the bulk of the phage protein appeared to have no further function and this suggested (but does not prove – that had to await more rigorous experiments with purified nucleic acid genomes) that the nucleic acid is the carrier of viral heredity. The transfer of the phage nucleic acid from its protein coat to the bacterial cell upon infection also accounts for the existence of the eclipse period during the early stages of intracellular virus development, since the nucleic acid on its own cannot normally infect a cell (Fig. 1.3).

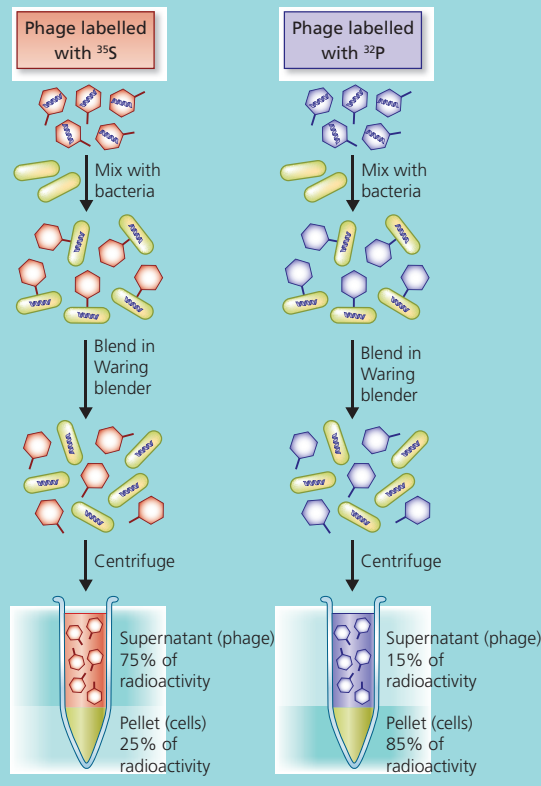

Fig. 1.3 The Hershey-Chase experiment proving that DNA (labelled with ^{32}P) is the genetic material of bacteriophage T2.

particles which are morphologically mature and fully infectious (see Chapter 12). When particles of two different strains (differing in the symptoms produced in the host plant) were each disassociated and the RNA of one reassociated with the protein of the other, and vice versa, the properties of the virus which was propagated when the resulting 'hybrid' particles were used to infect host plants were always those of the parent virus from which the RNA was derived (Fig. 1.4).

The ultimate proof that viral nucleic acid is the genetic material came from numerous observations that, under special circumstances, purified viral nucleic acid is capable of initiating infection, albeit with a reduced efficiency. For example, in 1956 Gierer and Schramm, and Fraenkel-Conrat independently showed that the purified RNA of tobacco mosaic virus can be infectious, provided precautions are taken to protect it from inactivation by ribonuclease. An extreme example is the causative agent of potato spindle tuber disease which lacks any protein component and consists solely of RNA. Because such agents have no protein coat, they cannot be called viruses and are referred to as *viroids*.

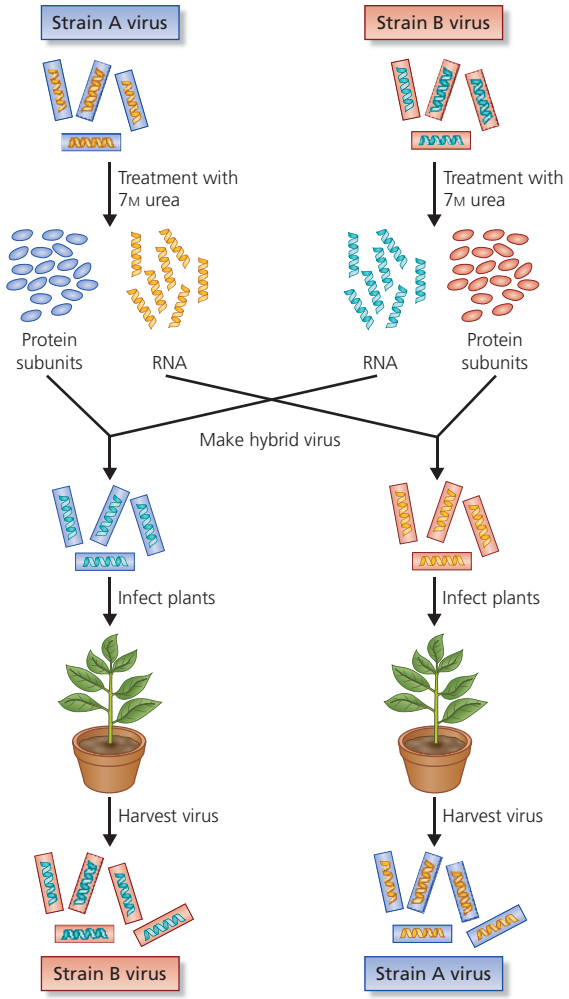

Fig. 1.4 The experiment of Fraenkel-Conrat and Singer which proved that RNA is the genetic material of tobacco mosaic virus.

Synthesis of macromolecules in infected cells

Following introduction of the virus genetic material into the cell, the next phase of the replication cycle is the synthesis of new macromolecules that play a role in the replication process and/or find their way into the next generation of virus particles. The discovery in 1953, by Wyatt and Cohen, that the DNA of the T-even bacteriophages T2, T4 and T6 contains hydroxymethylcytosine (HMC) instead of cytosine made it possible for Hershey, Dixon and Chase to examine infected bacteria for the presence of phage-specific DNA at various stages of intracellular growth. DNA was extracted from T2-infected *E. coli* at different times after the onset of phage growth, and analyzed for its content of HMC. This provided an estimate of the number of phage equivalents of HMC-containing DNA present at any time, based on the total nucleic acid and relative HMC content of the intact T2 phage particle. The results showed that, with T2, synthesis of phage DNA commences about 6 minutes after

infection and the amount present then rises sharply, so that by the time the first infectious particles begin to appear 6 minutes later there are 50–80 phage equivalents of HMC. Thereafter, the numbers of phage equivalents of DNA and of infectious particles increase linearly and at the same rate up until lysis, and continue to rise even if lysis is delayed beyond the normal burst time.

Hershey and his co-workers also studied the synthesis of phage protein, which can be distinguished from bacterial protein by its interaction with specific antibodies. During infection of *E. coli* by T2 phage, protein can be detected about 9 minutes after the onset of the latent period, i.e. after DNA synthesis begins and before infectious particles appear. A few minutes later there are approximately 30–40 phages inside the cell. Whereas the synthesis of viral protein starts about 9 minutes after the onset of the latent period, it was shown by means of pulse–chase experiments that the uptake of ^{35}S into intracellular protein is constant from the start of infection. A small quantity (a pulse) of ^{35}S (as sulphate) was added to the medium at different times after infection and was followed shortly after by a vast excess of unlabelled sulphate (chase) to stop any further incorporation of label. When the pulse was made from the 9th minute onward, the label could be chased into material identifiable by its reaction with antibody (i.e. serologically) as phage coat protein. However, if the pulse was made before 9 minutes of infection, although it could still be chased into protein and was non-bacterial, it did not react with antibodies to phage structural proteins. This early protein comprises mainly virus-specified enzymes that are concerned with phage replication but are not incorporated into phage particles. The concept of early and late, non-structural and structural viral proteins is discussed in Chapter 10.

These classical experiments are typical only of head-tail phages (see Section 2.5) infecting *E. coli* under optimum growth conditions. *E. coli* is normally found in the anaerobic environment of the intestinal tract, and it is doubtful that it grows with its optimal doubling time of 20 minutes under natural conditions. Other bacterial cells grow more slowly than *E. coli* and their viruses have longer multiplication cycles.

1.5 Multiplication of bacterial and animal viruses is fundamentally similar

The growth curves and other experiments described above have been repeated with many animal viruses with essentially similar results. Both bacterial and animal viruses attach to their target cell through specific interactions with cell surface molecules. Like the T4 bacteriophage, the genomes of some animal viruses (e.g. HIV-1) enter the cell and leave their coat proteins on the outside. However, with most animal viruses, some viral protein, usually from inside the particle, enters the cell in association with the viral genome. In fact, it is now known that some phage protein enters the bacterial cells with the phage genome. Such proteins are essential for genome replication. Many other animal viruses behave slightly differently, and after attachment are engulfed by the cell membrane and taken into the cell inside a vesicle. However, strictly speaking this virus has not yet entered the cell cytoplasm, and is still outside the cell. The virus genome gains entry to the cytoplasm through the wall of the vesicle, when the particle is stimulated to uncoat. Again, the outer virion proteins stay in the vesicle – i.e. outside the cell. Animal viruses go through the same stages of eclipse, and virus assembly from constituent viral components with linear kinetics, as bacterial viruses. Release of progeny virions may happen by cell lysis (although this is not an enzymatic

process as it is with some bacterial viruses), but frequently virus is released without major cell damage. The cell may die later, but death of the cell does not necessarily accompany the multiplication of all animal viruses. One major difference in the multiplication of bacterial and animal virus is that of time scale – animal virus growth cycles take in the region of 5–15 hours for completion.

1.6 Viruses can be manipulated genetically

One of the easiest ways to understand the steps involved in a particular reaction within an organism is to isolate mutants which are unable to carry out that reaction. Like all other organisms, viruses sport mutants in the course of their growth, and these mutations can affect all properties including the type of plaque formed, the range of hosts which the virus can infect, and the physico-chemical properties of the virus. One obvious caveat is that many mutations will be lethal to the virus and remain undetected. This problem was overcome in 1963 by Epstein and Edgar and their collaborators with the discovery of *conditional lethal mutants*. One class of these mutants, the *temperature-sensitive mutants*, was able to grow at a lower temperature than normal, the *permissive temperature*, but not at a higher, *restrictive* temperature at which normal virus could grow. Another class of conditional lethal mutants was the *amber* mutant. In these mutants a genetic lesion converts a codon within transcribed RNA into a triplet which terminates protein synthesis. They can only grow on a *permissive* host cell, which has an amber-suppressor transfer RNA (tRNA) that can insert an amino acid at the mutation site during translation.

The drawback to conditional lethal mutants is that mutation is random, but the advent of *recombinant DNA technology* has facilitated controlled mutagenesis, known as reverse genetics, at least for those viruses for which infectious particles can be reconstituted from cloned genomic DNA or cDNA (DNA that has been transcribed from RNA) inserted into a plasmid vector. This process is described in Section 5.7.

1.7 Properties of viruses

With the assumption that the features of virus growth described above for particular viruses are true of all viruses, it is possible to compare and contrast the properties of viruses with those of their host cells. Whereas host cells contain both types of nucleic acid, DNA and RNA, each virus contains only one type. However, just like their host cells, viruses have their genetic information encoded in nucleic acid. Another difference is that the virus is reproduced solely from its genetic material, whereas the host cell is reproduced from the integrated sum of its components. Thus, the virus never arises directly from a pre-existing virus, whereas the cell always arises by division from a pre-existing cell. The experiments of Hershey and his collaborators showed quite clearly that the components of a virus are synthesized independently and then assembled into many virus particles. In contrast, the host cell increases its constituent parts, during which the individuality of the cell is continuously maintained, and then divides and forms two cells. Finally, viruses are incapable of synthesizing ribosomes, and depend on pre-existing host cell ribosomes for synthesis of viral proteins. These features clearly separate viruses from all other organisms, even *Chlamydia* species, which for many years were considered to be intermediate between bacteria and viruses.

1.8 Origin of viruses

The question of the origin of viruses is a fascinating topic; as so often happens when hard evidence is scarce, discussion can be heated but often not illuminating. There are two popular theories: viruses are either degenerate cells or vagrant genes. Just as fleas are descended from flies by loss of wings, viruses may be derived from pro- or eukaryotic cells that have dispensed with many of their cellular functions (*degeneracy*). Alternatively, some nucleic acid might have been transferred accidentally into a cell of a different species (e.g. through a wound or by sexual contact) and, instead of being degraded as would normally be the case, might have survived and replicated (*escape*). Despite decades of discussion and argument there are no firm indications if either, or both, of these theories are correct. Rapid sequencing of viral and cellular genomes is now providing data for computer analysis that are giving an ever-better understanding of the relatedness of different viruses. However, while such analyses may identify, or more commonly infer, the progenitors of a virus, they cannot decide between degeneracy and escape. It is unlikely that all currently-known viruses have evolved from a single progenitor. Rather, viruses have probably arisen numerous times in the past by one or both of the mechanisms outlined above.

Key points

- Viruses are obligate intracellular parasites.
- It is likely that every living organism on this planet is infected by a species-specific range of viruses.
- Viruses multiply by assembling many progeny particles from a pool of virus-specified components, whereas cells multiply by binary fission.
- Viruses have probably originated independently many times.

Further reading

Flint, S. J., Enquist, L. W., Racaniello, V. R., Skalka, A. M. 2008. *Principles of Virology: Molecular Biology, Pathogenesis, and Control*, 3rd edn. ASM Press, Washington DC.

Hull, R. 2013. *Plant Virology*, 5th edn. Academic Press, San Diego.

Knipe, D. M., Howley, P. M. (2007). *Field's Virology*, 5th edn. Lippincott Williams & Wilkins, Philadelphia.

Maclachlan, N. J., Dubovi, E. J. 2010. *Fenner's Veterinary Virology*, 4th edn. Academic Press, San Diego.

Mahy, B. W. J., van Regenmortel, M. H. V. 2008. *Encyclopedia of Virology*, 3rd edn. Academic Press, San Diego.

Richman, D. D., Whitley, R. J., Hayden, F. G. 2009. *Clinical Virology*, 3rd edn. ASM Press, Washington DC.

Zuckerman, A. J., Banatvala, J., Griffiths, P. D., Schoub, B., Mortimer, P. 2009. *Principles and Practice of Clinical Virology*, 6th edn. John Wiley & Sons, Chichester.