CHAPTER 1

Viruses and Their Importance

CHAPTER 1 AT A GLANCE

Viruses infect:

- Humans

Smallpox[1]

- Other vertebrates

Bluetongue virus-infected sheep[2]

- Invertebrates

Tipula sp. larvae (leatherjackets) infected with invertebrate iridescent virus 1

- Plants

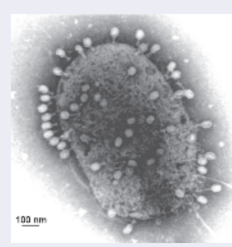
Delayed emergence of potato caused by tobacco rattle virus infection[3]

Damaged potato (spraing) caused by tobacco rattle virus infection[3]

- Fungi

Mushroom virus X[4]

- Bacteria



Escherichia coli cell with phage T4 attached[5]

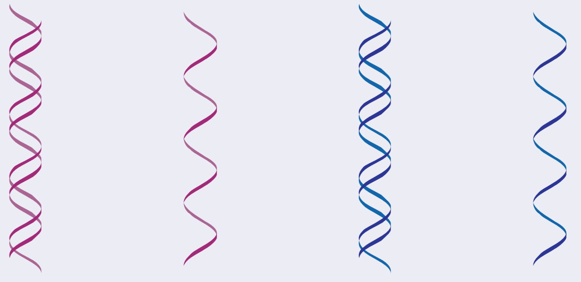
CHAPTER 1 AT A GLANCE (*continued*)

Some viruses are useful:
- Phage typing of bacteria
- Sources of enzymes
- Pesticides
- Anti-bacterial agents
- Anti-cancer agents
- Gene vectors

Viruses are parasites; they depend on cells for molecular building blocks, machinery, and energy.

Virus particles are small; dimensions range approx. from 20–400 nm.

A virus genome is composed of one of the following:



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[1]World Health Organization.
[2]From Umeshappa *et al*. (2011) *Veterinary Immunology and Immunopathology*, 141, 230. Reproduced by permission of Elsevier and the authors.
[3]MacFarlane and Robinson (2004) Chapter 11, *Microbe-Vector Interactions in Vector-Borne Diseases*, 63rd Symposium of the Society for General Microbiology, Cambridge University Press. Reprinted with permission.
[4]University of Warwick.
[5]Cornell Integrated Microscopy Center.

1.1 VIRUSES ARE UBIQUITOUS ON EARTH

Viruses infect all cellular life forms: eukaryotes (vertebrate animals, invertebrate animals, plants, fungi) and prokaryotes (bacteria and archaea). The viruses that infect bacteria are often referred to as bacteriophages, or phages for short.

The presence of viruses is obvious in host organisms showing signs of disease. Many healthy organisms, however, are hosts of non-pathogenic virus infections, some of which are active, while some are quiescent. Furthermore, the genomes of many organisms contain remnants of ancient virus genomes that integrated into their host genomes long ago. As well being present within their hosts, viruses are also found in soil, air and water. Many aqueous environments contain very high concentrations of viruses that infect the organisms that live in those environments.

There is a strong correlation between how intensively a species is studied and the number of viruses found in that species. Our own species is the subject of most attention as we have a vested interest in learning about agents that affect our health. It is not surprising that there are more viruses known that infect mankind than any other species, and new human viruses continue to be found. The intestinal bacterium *Escherichia coli* has also been the subject of much study and many viruses have been found in this species. If other species received the same amount of attention it is likely that many would be found to be hosts to similar numbers of viruses.

It is undoubtedly the case that the viruses that have been discovered represent only a tiny fraction of the viruses on the Earth. Most of the known plants, animals, fungi, bacteria and archaea have yet to be investigated for the presence of viruses, and new potential hosts for viruses continue to be discovered. Furthermore, the analysis of DNA from natural environments points to the existence of many bacterial species that have not yet been isolated in the laboratory; it is likely that these "non-cultivable bacteria" are also hosts to viruses.

1.2 REASONS FOR STUDYING VIRUSES

1.2.1 Some viruses cause disease

Viruses are important agents of many human diseases, ranging from the trivial (e.g. common colds) to the lethal (e.g. rabies), and viruses also play roles in the development of several types of cancer. As well as causing individuals to suffer, virus diseases can also affect the well-being of societies. Smallpox had a great impact in the past and AIDS is having a great impact today.

There is therefore a requirement to understand the nature of viruses, how they replicate and how they cause disease. This knowledge permits the development of effective means for prevention, diagnosis, and treatment of virus diseases through the production of vaccines, diagnostic reagents and anti-viral drugs. Vaccines, such as rotavirus and measles vaccines, have saved millions of lives and improved the quality of life for millions more. Smallpox has been eradicated as a result of vaccination. Anti-viral drugs, such as those used against HIV and herpes simplex virus, play major roles in the treatment of infectious disease. Medical applications therefore constitute major aspects of the science of virology.

Many viruses cause disease in domestic animals (such as cattle, sheep, dogs, poultry, fish, and bees) and in wild animals (such as red squirrels and seals). Vaccines are used to control some of these diseases, for example foot and mouth disease and bluetongue. Crop plants are also hosts to large numbers of viruses, such as rice yellow mottle virus and cucumber mosaic virus, a virus with a very wide host range. These, and other plant viruses, can cause devastating outbreaks of disease in crop plants, with significant impact on the quantity and quality of food produced.

Another area where viruses can cause economic damage is in those industries where the products result from bacterial fermentation. In the dairy industry phages can destroy the lactic acid bacteria used to produce cheese, yogurt, and other milk products, while other phages can destroy *Corynebacterium* species used in the industrial production of amino acids.

1.2.2 Some viruses are useful

Some viruses are studied because they have useful current or potential applications.

- *Phage typing of bacteria*. Some groups of bacteria, such as some *Salmonella* species, are classified into strains on the basis of the spectrum of phages to which they are susceptible. Identification of the phage types of bacterial isolates can provide useful epidemiological information during outbreaks of disease caused by these bacteria.
- *Sources of enzymes*. A number of enzymes used in molecular biology are virus enzymes. Examples include reverse transcriptases from retroviruses and RNA polymerases from phages.
- *Pesticides*. Some insect pests are controlled with baculoviruses, and myxoma virus has been used to control rabbits.
- *Anti-bacterial agents*. In the mid-twentieth century phages were used to treat some bacterial infections in humans. Interest waned with the discovery of antibiotics, but has been renewed with the emergence of antibiotic-resistant strains of bacteria.
- *Anti-cancer agents*. Genetically modified strains of viruses, such as herpes simplex virus and vaccinia virus, are being investigated for treatment of cancers. These strains have been modified so that they

are able to infect and destroy specific tumor cells, but are unable to infect normal cells.

- *Gene vectors for protein production*. Viruses, such as certain baculoviruses and adenoviruses, are used as vectors to take genes into animal cells growing in culture. This technology is used to make cells produce useful proteins, such as vaccine components. Some genetically modified cells are used for mass production of proteins.
- *Gene vectors for treatment of genetic diseases*. Children with the genetic disease Severe Combined Immunodeficiency (baby in the bubble syndrome) have been successfully treated using retroviruses as gene vectors. The viruses introduced into the children's stem cells a non-mutated copy of the mutated gene (Section 17.5).

1.2.3 Virus studies have contributed to knowledge

Much basic knowledge of molecular biology, cell biology, and cancer has been derived from studies with viruses. Here are a few examples.

- A famous experiment carried by Alfred Hershey and Martha Chase, and published in 1952, used phage T2 and *E. coli* to provide strong evidence that genetic material is composed of DNA.
- The first enhancers to be characterized were in genes of simian virus 40 (SV40).
- The first transcription factor to be characterized was the transplantation (T) antigen of SV40.
- The first nuclear localization signal of a protein was identified in the T antigen of SV40.
- Introns were discovered during studies of adenovirus transcription.
- The role of the cap structure at the 5′ end of eukaryotic messenger RNA (mRNA) was discovered during studies with vaccinia virus and a reovirus.
- The first internal ribosome entry site to be discovered was found in the RNA of poliovirus.
- The first RNA pseudoknot to be discovered was in the genome of turnip yellow mosaic virus.

1.3 THE NATURE OF VIRUSES

1.3.1 Viruses are small particles

Evidence for the existence of very small infectious agents was first provided in the late nineteenth century by two scientists working independently: Martinus Beijerinck in Holland and Dimitri Ivanovski in Russia. They made extracts from diseased plants, which we now know were infected with tobacco mosaic virus, and passed the extracts through fine filters. The filtrates contained an agent that was able to infect new plants, but no bacteria could be cultured from the filtrates. The agent remained infective through several transfers to new plants, eliminating the possibility of a toxin. Beijerinck called the agent a "virus" and the term has been in use ever since.

At around the same time, Friedrich Löeffler and Paul Frosch transmitted foot and mouth disease from animal to animal in inoculum that had been highly diluted. A few years later Walter Reed and James Carroll demonstrated that the causative agent of yellow fever is a filterable agent.

Figure 1.1 gives some indication of the size of these agents, which are known as virus particles or virions. The virion of a herpesvirus, which is a fairly large virus, is about ten million times smaller than a large balloon, while the balloon is smaller than the Earth by the same factor. The virions of most viruses are too small to be seen with a light microscope and can be seen only with an electron microscope (Figure 1.2).

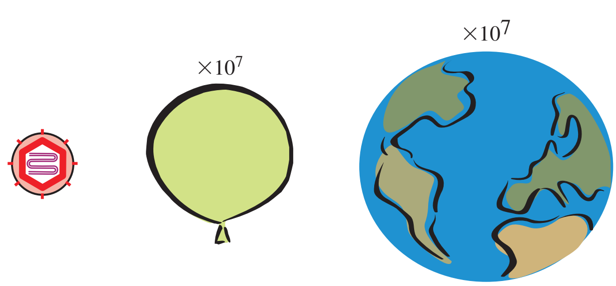

Figure 1.1 Comparative sizes of a herpesvirus particle, a balloon, and the Earth. A large balloon is about ten million times larger than a herpesvirus particle, while the Earth is larger than the balloon by the same factor.

Figure 1.2 **Transmission electron microscope.** This is a microscope in which the image is formed by electrons transmitted through the specimen.
Source: Photograph courtesy of JEOL.

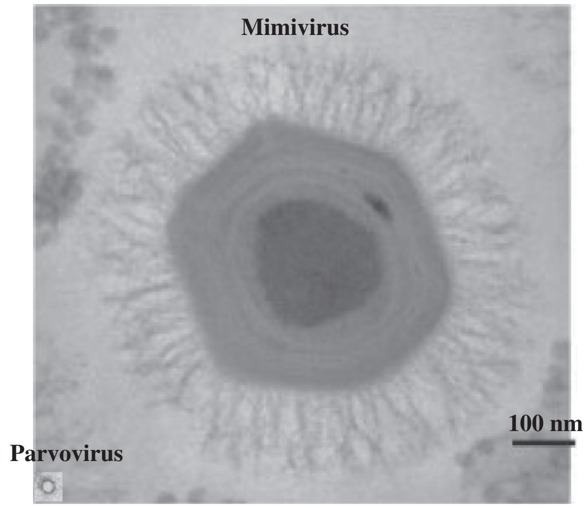

Figure 1.3 **Virions of mimivirus, one of the largest viruses, and a parvovirus, one of the smallest viruses.**
Source: Electron micrograph of mimivirus from Claverie *et al.* (2009) *Journal of Invertebrate Pathology*, 101, 172. Reproduced by permission of Elsevier and the authors. Electron micrograph of parvovirus by permission of Professor M. Stewart McNulty and The Agri-Food and Biosciences Institute.

The units in which virions are normally measured are nanometers ($1\,nm = 10^{-9}\,m$). Although virions are very small, their dimensions cover a large range. Amongst the smallest are parvoviruses (Figure 1.3), with diameters about 20 nm, while the megavirus and mimivirus (*mi*crobe-*mi*micking virus), isolated from amebae, are amongst the largest.

Virology is therefore concerned with very small particles, though often with very large numbers of those particles! A concentrated suspension of virions might contain 10^{12} virions/ml. A single virus-infected cell might produce 10^5 virions. A person infected with HIV might produce 10^{11} virions in a day.

Virions are not cells. They do not contain organelles, except for the virions of arenaviruses, which contain cell ribosomes that were packaged when the virions were assembled.

1.3.2 Viruses have genes

The virion contains the genome of the virus. Whereas the genomes of cells are composed of double-stranded DNA, there are four possibilities for a virus genome:

- double-stranded DNA (dsDNA);
- single-stranded DNA (ssDNA);
- double-stranded RNA (dsRNA);
- single-stranded RNA (ssRNA).

The genome is enclosed in a protein coat known as a capsid. The genome plus the capsid, plus other components in many cases, constitute the virion. A major function of the capsid is to protect the genome until it can be delivered into a cell in which it can replicate.

Generally, virus genomes are much smaller than cell genomes and the question arises as to how viruses encode all their requirements in a small genome. Viruses achieve this in a number of ways.

- *Viruses use host cell proteins*. The genomes of large viruses duplicate some functions of the host cell, but the small viruses rely very heavily on host cell functions. There is, however, one function that an RNA virus must encode, no matter how small its genome. That function is an RNA polymerase, because cells do not encode enzymes that can replicate virus RNA. A significant proportion of an RNA virus genome is taken up with the gene for an RNA polymerase.

- *Viruses code efficiently*. There may be overlapping genes and genes encoded within genes. The small genome of hepatitis B virus is a good example (see Section 19.6). In viruses most, or all, of the genome encodes proteins, in contrast to mammals where only about 1.5% of the genome encodes proteins.

- *Many virus proteins are multifunctional.* Some virus proteins have multiple enzyme activities, for example the rhabdovirus L protein replicates RNA, caps and polyadenylates mRNA, and phosphorylates another virus protein. Some virus proteins can bind to cell proteins with roles in immune responses, and hence interfere with immunity of the host.

1.3.3 Viruses are parasites

Viruses differ from cells in the way in which they multiply. A new cell is always formed directly from a pre-existing cell, but a new virion is never formed directly from a pre-existing virion. New virions are formed by a process of replication, which takes place inside a host cell and involves the synthesis of components followed by their assembly into virions.

Viruses are therefore parasites of cells, and are dependent on their hosts for most of their requirements, including:

- building-blocks such as amino acids and nucleosides;
- protein-synthesizing machinery (ribosomes);
- energy, in the form of adenosine triphosphate.

A virus modifies the intracellular environment of its host in order to enhance the efficiency of the replication process. It does this by producing proteins, and in some cases small RNAs, that interact with cell components. It has been demonstrated that the proteins of a virus can interact with hundreds of host proteins in an infected cell. The expression of thousands of host genes can be affected, with some genes up-regulated and some genes down-regulated.

Virus-induced modifications to a host cell might include the formation of new membranous structures or a reduced immune response. Some large phages of photosynthetic bacterial hosts encode proteins that enhance photosynthesis, thereby probably boosting the yields of virus from the cells.

A point has now been reached where the nature of viruses can be summarized in a concise definition (see the box).

VIRUS DEFINITION

A virus is a very small, non-cellular parasite of cells. Its genome, which is composed of either DNA or RNA, is enclosed in a protein coat.

1.3.4 Some viruses are dependent on other viruses

Some viruses, known as satellite viruses, are unable to replicate unless the host cell is infected with a second virus, referred to as a helper virus. The helper virus provides one or more functions missing from the satellite virus, thereby enabling the latter to complete its replication cycle. Examples are given in Table 1.1

1.3.5 Are viruses living or nonliving?

"Viruses belong to biology because they possess genes, replicate, evolve, and are adapted to particular hosts, biotic habitats, and ecological niches. However, . . . they are nonliving infectious entities that can be said, at best, to lead a kind of borrowed life."

Marc van Regenmortel and Brian Mahy (2004)

"It's life, Jim, but not as we know it!"

Dr McCoy speaking to Captain Kirk of the Starship Enterprise, *Star Trek*

Table 1.1 Examples of satellite/helper viruses

Type of host	Satellite virus	Genome	Helper virus	Genome
Animal	Hepatitis delta virus	ssRNA	Hepatitis B virus	dsDNA
	Adeno-associated viruses	ssDNA	Adenovirus	dsDNA
Plant	Satellite tobacco necrosis virus	ssRNA	Tobacco necrosis virus	ssRNA
Ameba	Sputnik	dsDNA	Mimivirus	dsDNA
Bacterium	Enterobacteria phage P4	dsDNA	Enterobacteria phage P2	dsDNA

There is an ongoing debate as to whether viruses are living or nonliving; the view taken depends on how life is defined. Viruses have genes and when they infect cells these genes are replicated, so in this sense viruses are living. They are, however, very different to cellular life forms, so Dr McCoy's stock phrase (see the box) on finding new life forms in the galaxy could be applied to viruses. When viruses are outside their host cells they exist as virions, which are inert, and could be described as nonliving, but viable bacterial spores are inert and are not considered to be nonliving. You might form your own view as to whether viruses are living or nonliving as you progress through this book.

When Beijerinck selected the word "virus" he chose the Latin word for poison. This term has now been in use for over a century and virology has developed into a huge subject. More recently, the term virus has acquired further meanings. Computers are threatened by *infection* with viruses that can be found *in the wild* once they have been *released* by their authors. These viruses are *specific* for certain file types. *Infected* files may be put on several web sites and a virus *epidemic* may ensue. Another use of the term virus is exemplified in John Humphrys' book Lost For Words, in which he talks about the *deadly* virus of management-speak *infecting* language. All the italicized terms in this paragraph are also used in the context of the viruses that are the subject of this book.

1.4 THE REMAINDER OF THE BOOK

Having outlined the nature of viruses and why they are important, the remainder of the book will examine many aspects of fundamental and applied virology. The early chapters cover principles, such as the structure of virions, virus replication, and the classification of viruses. There are then ten chapters devoted to reviews of particular groups of viruses, where both principles and applications of virology are covered. Towards the end of the book we consider specific applications of virology, including viral vaccines and anti-viral drugs. The final chapter is devoted to prions, which are not viruses!

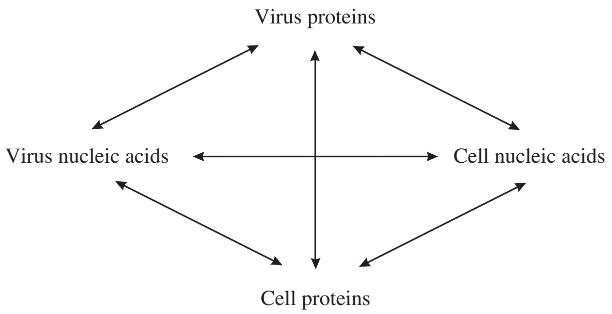

Figure 1.4 Interactions between virus molecules and cell molecules.

It is important to point out that much of virology is concerned with characteristics of the proteins and nucleic acids of viruses, and with interactions between these molecules and the proteins and nucleic acids of cells (Figure 1.4). Most of these interactions rely on specific binding between the molecules. We shall also be discussing cellular structures, and processes such as transcription, translation, and DNA replication. A good background in molecular biology and cell biology is therefore essential; some useful sources of information for plugging any gaps can be found under *Sources of further information*.

LEARNING OUTCOMES

After review of this chapter you should be able to:

- discuss reasons for studying viruses;
- explain how viruses differ from cellular organisms;
- define the term "virus."

SOURCES OF FURTHER INFORMATION

Cell biology and molecular biology books

Alberts, B. *et al*. (2008) *Molecular Biology of the Cell*, 5th edition, Garland
Bolsover, S. R. *et al*. (2011) *Cell Biology: A Short Course*, 3rd edition, Wiley-Blackwell

Karp, G. (2010) *Cell Biology*, 6th edition, Wiley
Lodish, H. F. *et al*. (2008) *Molecular Cell Biology*, 6th edition, Freeman
Pollard, T. D. and Earnshaw, W. C. (2008) *Cell Biology*, 2nd edition, Saunders
Watson, J. D. *et al*. (2007) *Molecular Biology of the Gene*, 6th edition, Pearson
Weaver, R. F. (2011) *Molecular Biology*, 5th edition, McGraw-Hill

Historical paper

Hershey, A. D. and Chase, M. (1952) Independent functions of viral protein and nucleic acid in growth of bacteriophage. *Journal of General Physiology*, 36, 39–56

Recent papers

Breitbart, M. and Rohwer, F. (2005) Here a virus, there a virus, everywhere the same virus? *Trends In Microbiology*, 13, 278–284
Enquist, L. W. (2009) Virology in the 21st century. *Journal of Virology*, 83, 5296–5308
Hunter, W. *et al*. (2010) Large-scale field application of RNAi technology reducing Israeli acute paralysis virus disease in honey bees (*Apis mellifera*, Hymenoptera: Apidae). *PLoS Pathogens*, 6 (12): e1001160
Kutateladze, M. and Adamia, R. (2010) Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends in Biotechnology*, 28, 591–595
Roossinck, M. J. (2011) The good viruses: viral mutualistic symbioses. *Nature Reviews Microbiology*, 9, 99–108
Scholthof, K.-B. G. *et al*. (2011) Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12, 938–954
van Regenmortel, M. H. V. and Mahy, B. W. J. (2004) Emerging issues in virus taxonomy. *Emerging Infectious Diseases*, 10 (1): http://www.cdc.gov/ncidod/eid/vol10no1/03-0279.htm
Young, L. S. *et al*. (2006) Viral gene therapy strategies: from basic science to clinical application. *Journal of Pathology*, 208, 299–318