

Introduction to Virology

Flint et al. Principles of Virology (ASM), Chapter 1 and 2
Wagner & Hewlett. Basic Virology (Blackwell). Chapter 10

Historical Perspective

Many viruses have co-evolved with mammals and other animals over long periods of time. Examples of such viruses are herpesviruses, which have been traced back to fish and birds, as well as mammals. It is thought that herpesviruses have existed for two hundred million years or longer, and that they have infected humans since the early times of our speciation. Other viruses have entered human populations only recently, due to changes in agriculture (use of domestic animals), population dynamics (urbanization), migration of populations, commerce and changes in the environment. Examples of these agents include measles virus and HIV-1.

Vaccines

Attempts to control smallpox have existed for almost one thousand years. The early method was called **variolation**, and involved the inoculation of healthy individuals with material from a smallpox pustule, by scratching of the arm. This crude method was effective, but was accompanied by serious side effects including disseminated skin lesions and even death in about 1% of cases. However, this was preferable to the very high fatality rate of the natural infection (25% or so, and more in children).

The concept of **vaccination** (vacca = cow) arose in the 1790s, when Jenner made the observation that milk maids exposed to cowpox were protected against smallpox. Jenner showed that the deliberate inoculation of a boy with cowpox virus resulted in protection from smallpox. The cowpox vaccine was propagated for many years in humans, before being grown in animals, but at some point the cowpox virus unknowingly became replaced by the virus now known as vaccinia. Vaccinia virus has been found in “cowpox” vaccines dating from the 1870s, and it is not clear how the virus arose – although it may have arisen in horses.

The derivation of deliberately **attenuated** vaccines did not occur until the work of Pasteur in the 1880s, who serially passaged rabies virus in rabbits in order to derive a weakened (attenuated) strain that still elicited protective immunity but which failed to cause disease.

Discovery of viruses

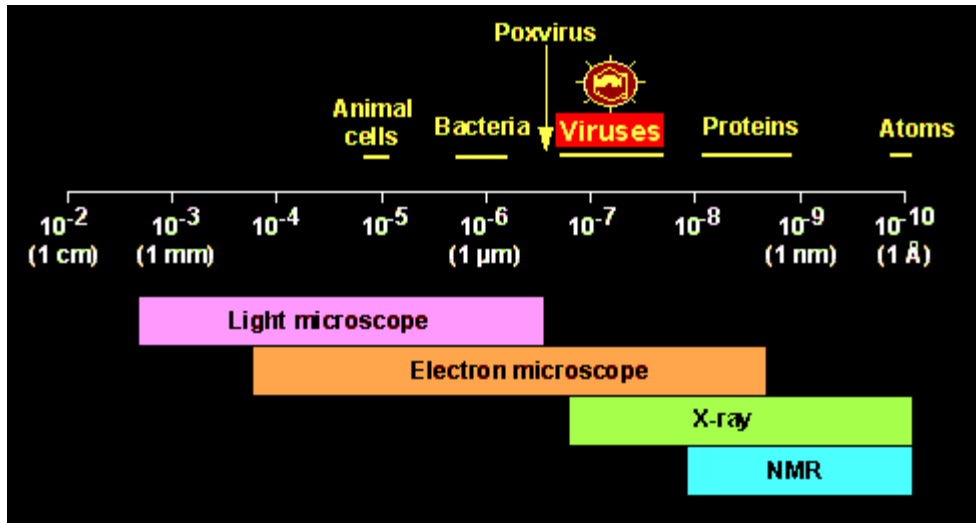
In the 1890s, scientists discovered that the agent which caused tobacco mosaic disease was a filterable agent smaller than bacteria. By the early 1900s, additional viruses had been identified, including viruses which caused tumors in chickens (e.g., the Rous sarcoma virus) as well as yellow fever virus (the first human virus to be discovered, in 1901).

Definitive Properties of Viruses

Perhaps the defining single feature of viruses is that they are **obligate intracellular molecular parasites**. A more complete list of the defining properties of viruses is as follows:

1. Viruses are obligate intracellular molecular parasites, which are very small and infectious.
2. The virus genome is composed either of DNA or RNA.
3. The virus genome directs the synthesis of virion components within an appropriate host cell.
4. Progeny virus particles are produced by the assembly of newly made viral components.
5. Progeny virus particles spread infection to new cells.

Size of viruses



Classification of Viruses

Classical virus classification schemes have been based on the consideration of four major properties of viruses:

1. The type of nucleic acid which is found in the virion (RNA or DNA)
2. The symmetry and shape of the capsid
3. The presence or absence of an envelope
4. The size of the virus particle

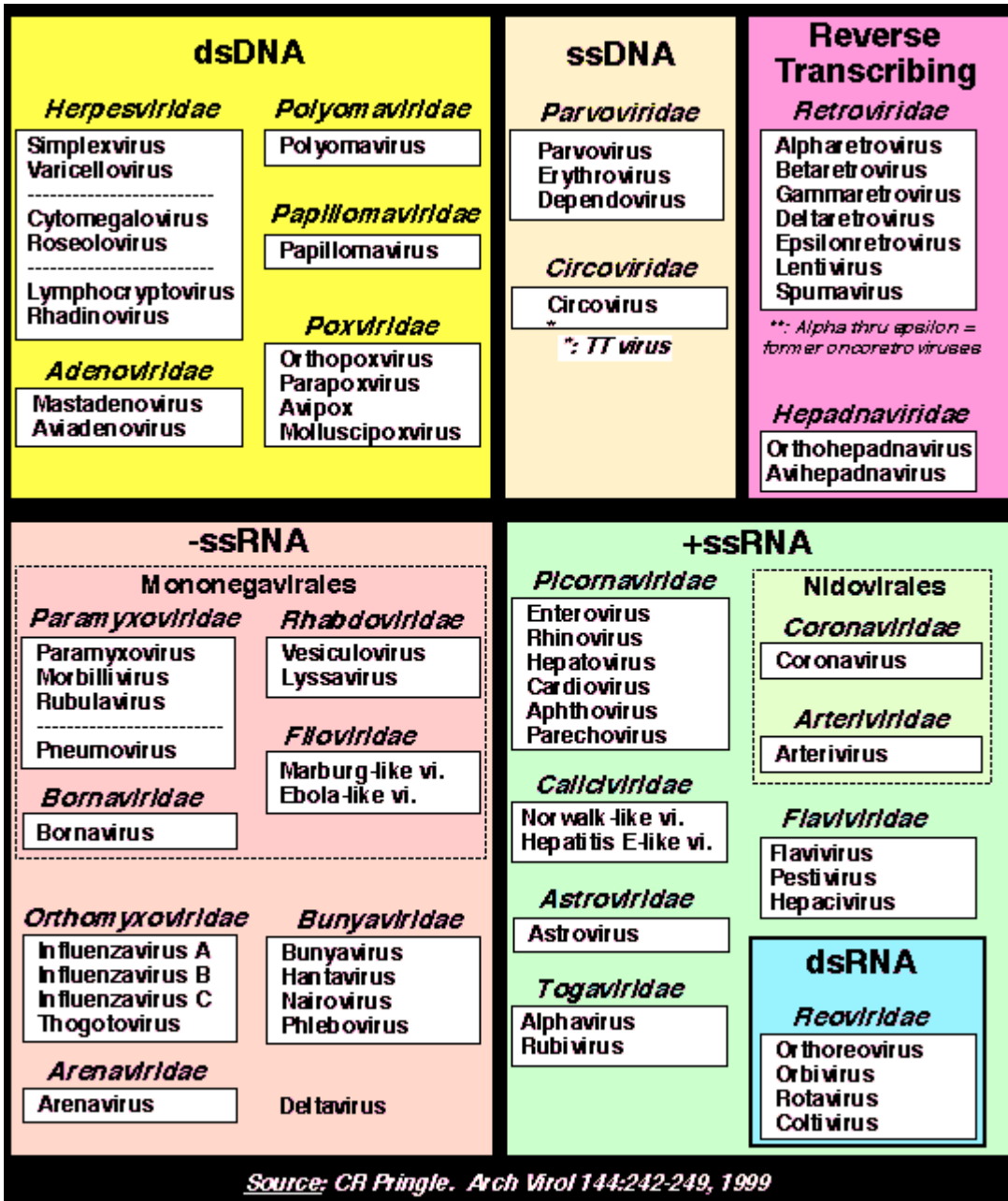
More recent classification systems adopted by the International Committee on Viral Taxonomy (ICTV) have really emphasized the viral genome as the primary determinant for viral taxonomy. Furthermore, there is a drift towards the use of **genomics** for virus classification – that is sequence analysis of the viral genome, and comparison to other known viral sequences.

The naming system for viruses that has been adopted by the ICTV is very useful for animal viruses, and is widely used. Latinized virus family names start with capital letters and end with the suffix *-viridae* (e.g., *Herpesviridae*). These formal names are often used interchangeably with the common names for viruses (e.g., herpesviruses).

Levels of taxonomy

Taxonomic level	Suffix (comment)	Example
Order	-virales (a group of related families)	<i>Mononegavirales</i>
Family	-viridae	<i>Paramyxoviridae</i>
Subfamily	-virinae	<i>Paramyxovirinae</i>

Genus	-virus	<i>Morbillivirus</i>
Species	(an individual virus)	<i>Measles virus</i>



Baltimore System for Virus Classification

The Baltimore system for virus classification is a system of classification which complements the ICTV classification system. It is especially useful for understanding viral replication strategies and will be discussed later.

Genetic Content of Viruses

DNA viruses: Almost all DNA viruses which infect animals contain double-stranded DNA. Exceptions include the *Parvoviridae* (e.g., parvovirus B19, adeno-associated virus) and the *Circoviridae* (these include the recently discovered TT virus, which may be related to the development of some cases of hepatitis).

RNA viruses: Almost all RNA viruses contain single-stranded RNA. Exceptions include the *Reoviridae* (e.g., rotaviruses) which contain double-stranded RNA. Other RNA viruses can be broadly subdivided as follows:

- **Viruses with positive strand (+) RNA genomes** – i.e., genomes of the same polarity as mRNA. Viruses in this category include picornaviruses and caliciviruses. In addition, retroviruses contain two copies of +RNA, although they replicate by a unique mechanism.
- **Viruses with negative strand (-) RNA genomes** – i.e., genomes of opposite polarity to mRNA. Viruses in this category all have helical capsids. Three members of the class are sufficiently closely related to comprise a distinct taxonomic order – the Mononegavirales (rhadboviruses, paramyxoviruses and filoviruses). The other (-) strand RNA viruses have segmented genomes (orthomyxoviruses have 8 segments while arenaviruses and bunyaviruses have either two or three segments, respectively. The arenaviruses and some bunyaviruses are also unique in that they possess **ambisense** genomes (i.e., their genomes contain both (+) and (-) strand RNAs).

PhysicoChemical Properties

Capsid (*sometimes referred to as nucleocapsid*): This is the protective protein shell surrounding the viral genome. Capsids are typically formed from a small number of protein subunits, which are assembled into repeating, symmetrical structures. The major classes of capsid symmetry are (1) **helical** (rod-like) and (2) **icosahedral** (sphere-like). Virus structure will be discussed in greater detail later, but suffice to say that virus shapes and sizes are highly diverse. The size of the capsid will, in large measure, dictate the amount of genetic material which can be packaged into the virus particle.

Envelope: Many animal viral envelopes are surrounded by a lipid bilayer, which is derived from the host cell membrane during the process of virus budding. These viral envelopes also contain virally-encoded proteins, which are often glycoproteins. These envelope proteins and glycoproteins often play a role in the processes of virus attachment and entry/uptake. **Important:** Envelopes are not present on all viruses, and that viruses which contain envelopes are usually **less stable** those that do not, e.g. herpes-viruses, which do and polio and human papillomaviruses (wart viruses), which do not.

Unifying Principles

All viruses employ in order to survive:

1. All viruses package their genomes inside a particle to ensure transfer from host to host.
2. All viruses can establish themselves in a host population, so as to ensure virus survival
3. The viral genome contains the information needed to initiate and complete an infectious cycle within a susceptible, permissive host cell. An infectious cycle includes attachment and entry/uptake, production of viral mRNA and proteins, genome replication and assembly and release of new particles.

Study of Viruses

Growing and maintaining viruses in the laboratory requires the ability to maintain cultures of cells which the virus can replicate itself in. In some cases, this is relatively easy to do, while in other cases (e.g., hepatitis C virus, human papillomaviruses) it can be quite difficult to get the virus to replicate in cell culture.

The culture of animal cells typically involves the use of culture medium containing salts, amino acids, vitamins, glucose, antibiotics, buffers and, usually, blood serum which provides a source of necessary cellular growth factors. For some cells, defined serum-free media have been developed, which contain specific growth factors, but in most cases, the addition of serum, with its complex mixture of nutritive factors, works best. Different cell types and cell lines have different media requirements.

All animal cells derive ultimately from living tissue. In some cases, these cells have been in culture for so long that they have long since ceased to resemble the tissue from which they were first isolated. **Continuous cell lines** of this kind are very convenient because they grow well and can be used to generate large amounts of virus. However, continuous cell lines are not useful for examining the effects of virus infection on cellular processes, since these cell lines are so different from the cells which the virus might normally infect *in vivo*. Thus, to study the effects of virus infection on cellular metabolism, differentiation, function and survival it is often best to use primary cells.

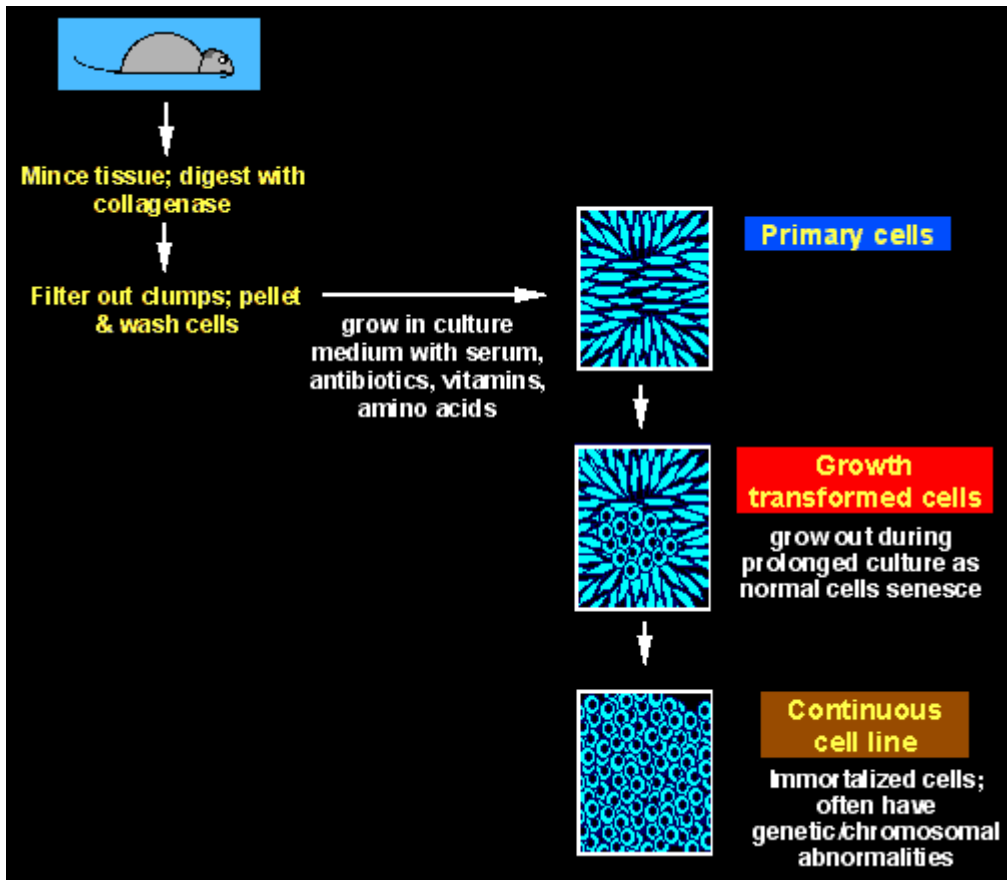
Cells with the properties of transformed cells can also be generated from tumors. In general, **tumor cells** have a phenotype that is broadly similar to that of continuous cell lines. Typically, tumor cells will generate tumors in the original host species, although this ability can be lost upon extensive passage.

Comparison of the properties of primary cells versus continuous cell lines or tumor cells

Property	Primary cells	Continuous cell lines or tumor-derived cell lines
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Derivation	Normal tissue	Tumor tissue, or by exposure of primary cells to a mutagen or transforming agent
Chromosomes	Normal	Aneuploid. Abnormal chromosomes, and/or altered chromosome numbers
Lifespan	Finite. After 5-20 generations, the cells will senesce and die.	Immortal. If fed, the cells will grow forever.
Serum dependence	Require high amounts of serum to grow.	Need little serum.
Contact inhibition	Cell growth is arrested when cells touch each other.	No contact inhibition of cell growth or movement
Anchorage dependence	Require adherence to a solid support for growth (<i>except for lymphoid cells</i>)	Grow in suspension
Differentiation state	Fully differentiated	De-differentiated
Reintroduction into animals	Can reintroduce into the original host species without causing a tumor	May cause tumors in the original host species

Comparison of primary cells versus growth transformed cells and continuous cell lines



Outcome of virus infection

Many viruses kill the cells they infect, and this can often be reflected by the detachment of the cells from the plastic surface to which they had previously been adherent. Visible changes in cells that are induced by viruses are referred to as **cytopathic effects (CPE)**. These may include:

1. Membrane blebbing
2. Formation of multinucleated giant cells, or fused cells, known as syncytia
3. Production of inclusion bodies (accumulations of viral proteins or virions) in the nucleus or cytoplasm
4. Rounding up and detachment of cells from the culture dish
5. Cell lysis

The rate at which CPE develop following virus infection is variable and depends upon the rate of virus replication in culture. Thus, cytomegalovirus (slow replicating) takes several weeks to cause CPE, while herpes simplex virus (fast replicating) causes CPE rapidly. Some viruses can replicate without causing obvious CPE (e.g., some retroviruses).