7 General Virology

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Definition

■ Viruses are complexes consisting of protein and an RNA or DNA genome. They lack both cellular structure and independent metabolic processes. They replicate solely by exploiting living cells based on the information in the viral genome. ■

Viruses are **autonomous infectious particles** that differ widely from other microorganisms in a number of characteristics: they have no cellular structure, consisting only of proteins and nucleic acid (DNA or RNA). They have no metabolic systems of their own, but rather depend on the synthetic mechanism of a living host cell, whereby the viruses exploit normal cellular metabolism by delivering their own genetic information, i.e., nucleic acid, into the host cell. The host cell accepts the nucleic acid and proceeds to produce the

Table 7.**1** Essential Characteristics of Viruses

Size	25 nm (picornavirus) to 250 × 350 nm (smallpox virus). Resolving power of a light microscope: 300 nm, bacteria: 500–5000 nm. The comparative sizes are illustrated in Fig. 7.**1**.
Genome	DNA or RNA. Double-stranded or single-stranded nucleic acid, depending on the species.
Structure	Viruses are complexes comprising virus-coded protein and nucleic acid; some viral species carry cell-coded components (membranes, tRNA).
Reproduction	Only in living cells. The virus supplies the information in the form of nucleic acids and in some cases a few enzymes; the cell provides the remaining enzymes, the protein synthesizing apparatus, the chemical building blocks, the energy, and the structural framework for the synthetic steps.
Antibiotics	Viruses are unaffected by antibiotics, but can be inhibited by interferon and certain chemotherapeutic agents.

Comparative Sizes of Viruses and Bacteria

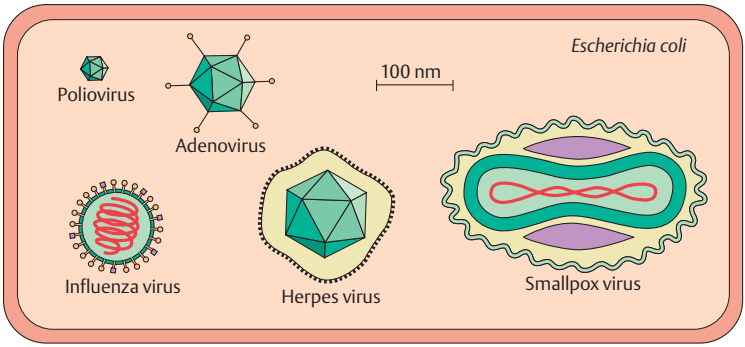


Fig. 7.**1** Different virus species are shown here to scale inside an *E. coli* bacterium.

components of new viruses in accordance with the genetic information it contains. One thus might call viruses "vagabond genes."

Viruses infect bacteria (so-called bacteriophages), plants, animals, and humans. The following pages cover mainly the human pathogen viruses (see Fig. 7.**1**). Table 7.**1** lists the essential characteristics of viruses.

Morphology and Structure

■ A mature virus particle is also known as a **virion**. It consists of either two or three basic components:

– A **genome** of DNA or RNA, double-stranded or single-stranded, linear or circular, and in some cases segmented. A single-stranded nucleic acid can have plus or minus polarity.
– The **capsid**, virus-coded proteins enclosing the nucleic acid of the virus and determining its antigenicity; the capsid can have a cubic (rotational), helical or complex symmetry and is made up of subunits called capsomers.
– In some cases an **envelope** (Fig. 7.**2**) that surrounds the capsid and is always derived from cellular membranes. ■

Genome. The viral genome is either DNA or RNA, and viruses are hence categorized as DNA or RNA viruses (see also p. 380). The nucleic acid of DNA viruses is usually double-stranded (ds) and linear or circular depending

Virus Particle Structure

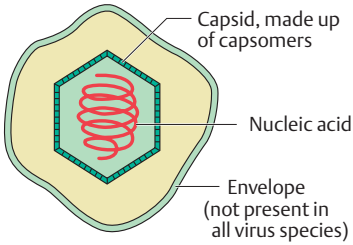


Fig. 7.**2**

on the family; the nucleic acid of RNA viruses is usually single-stranded (ss), with the exception of the reoviruses, and is also segmented in a number of virus families. Viruses with ssRNA are divided into two groups: if the RNA of the genome has the same polarity as the viral mRNA and can thus function directly as messenger RNA it is called a plus-strand (or positive-strand) or "sense" RNA strand and these viruses are *sense* or *plus-strand* viruses. If the genome RNA has the polarity opposite to that of the mRNA, and therefore cannot be translated into proteins until it has first been transcribed into a complementary strand, it is called a minus-strand (or negative-strand) or "antisense" RNA strand and the viruses are *antisense* or *minus-strand* viruses.

Capsid. The capsid (Fig. 7.**2**) is the "shell" of virus-coded protein that encloses the nucleic acid and is more or less closely associated with it. The combination of these two components is often termed the nucleocapsid, especially if they are closely associated as in the myxoviruses. The capsid is made up of subunits, the capsomers, the number of which varies but is specific and constant for each viral species. These are spherical or cylindrical structures composed of several polypeptides. The capsid protects the nucleic acid from degradation. In all except enveloped viruses, it is responsible for the attachment of the viruses to the host cell ("adsorption," see the chapter on replication, p. 384) and determines specific viral antigenicity.

Envelope. The envelope (Fig. 7.**2**), which surrounds the capsid in several virus families, is always dependent on cellular membranes (nuclear or cell membrane, less frequently endoplasmic reticulum). Both cell-coded and viral proteins are integrated in the membrane when these elements are transformed into the envelope, frequently in the form of "spikes" (or peplomers, Fig. 7.**3**). Enveloped viruses do not adsorb to the host cell with the capsid, but rather with their envelope. Removing it with organic solvents or detergents reduces the infectivity of the viruses ("ether sensitivity").

Other Components of Viral Particles

Various **enzymes**. Viruses require a number of different enzymes depending on genome type and mode of infection. In several virus species enzymes are a component of the virus particle, for example the neuraminidase required for invasion and release of myxoviruses. Other examples include nucleic acid polymerases such as the RNA-dependent RNA polymerases in antisense viruses, the DNA polymerases in smallpox viruses and the RNA-dependent DNA polymerase ("reverse transcriptase") in hepatitis B viruses and retroviruses (see p. 385f.).

Hemagglutinin. Some viruses (above all myxoviruses and paramyxoviruses) are capable of agglutinating various different human or animal erythrocytes. These viruses bear a certain surface protein (hemagglutinin) in their envelope that enables them to do this. The hemagglutination phenomenon can be made use of for quantitative viral testing or—in the hemagglutination inhibition test—for virus identification and antibody identification (p. 405ff.). In biological terms, hemagglutinin plays a decisive role in adsorption and penetration of the virus into the host cell.

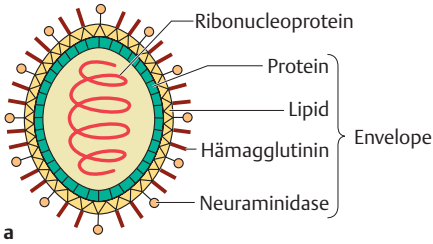
Structural Patterns

Cubic symmetry (rotational symmetry). Viruses with rotational symmetry are icosahedrons (polyhedrons with 20 equilateral triangular faces). The number of capsomers per virion varies from 32 to 252 and depends on the number of capsomers (two to six) making up one side of the equilateral triangle. The capsomers in a virion need not all be the same, either in their morphology, antigen make-up or biological properties. Purified icosahedral viruses can be crystallized, so that images of them can be obtained using the methods of radiocrystallography. A number of virus images have been obtained with this method at a resolution of 2 A.

Helical symmetry. Helical symmetry is present when one axis of a capsid is longer than the other. The nucleic acid and capsid protein are closely associated in the ribonucleoprotein (RNP), in which the protein is tightly arrayed around the nucleic acid strand. This RNA-protein complex is known as the nucleocapsid, which takes the form of a helix inside the viral envelope. Fig. 7.**3b** shows this in influenza virus the envelope of which has been partly removed and Fig. 7.**3a** illustrates these symmetries schematically.

Complex symmetry. Complex structural patterns are found in bacteriophages and the smallpox virus (see Fig. 7.**1**, right). T bacteriophages, for example, have an icosahedral head containing the DNA and a tubelike tail through which the DNA is injected into the host cell.

Viruses with Helical Symmetry



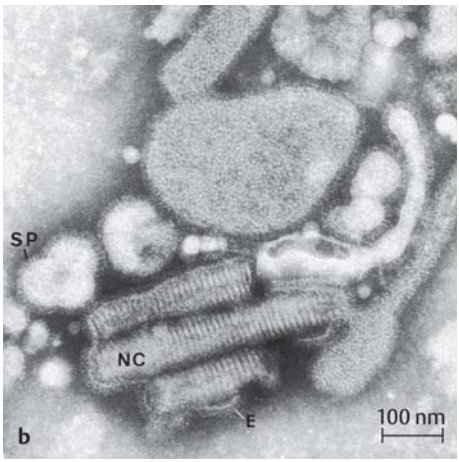


Fig. 7.3 **a** Schematic structure of a myxovirus. **b** Influenza viruses viewed with an electron microscope: The ribonucleoprotein spiral (nucleocapsid, NC) is visible inside the partially removed envelope (E). S = spikes.

Classification

The taxonomic system used for viruses is artificial (i.e., it does not reflect virus evolution) and is based on the following morphological and biochemical criteria:

- **Genome:** DNA or RNA genome (important basic differentiation of virus types!) as well as configuration of nucleic acid structure: single-stranded (ss) or double-stranded (ds); RNA viruses are further subclassified according to plus and minus polarity (p. 383f.).
- **Capsid symmetry:** cubic, helical, or complex symmetry.
- Presence or absence of an **envelope**.
- **Diameter** of the virion, or of the nucleocapsid with helical symmetry.

The origins and evolution of the viruses are still largely in the dark. In contrast to the taxonomic systems used to classify the higher forms of life, we are therefore unable to classify viruses in such evolutionary systems. An international nomenclature committee groups viruses according to various criteria and designates these groups, analogously to the higher forms, as families, genera, and species. Despite this element of "artificiality" in the system now in use, the groups appear to make biological sense and to establish order in the enormous variety of known viruses (see Table 7.**2**, based on publications by the International Committee on Taxonomy of Viruses).

Replication

- The steps in viral replication are as follows:
- **Adsorption** of the virus to specific receptors on the cell surface.
- **Penetration** by the virus and intracellular release of nucleic acid.
- **Proliferation** of the viral components: virus-coded synthesis of capsid and noncapsid proteins, replication of nucleic acid by viral and cellular enzymes.
- **Assembly** of replicated nucleic acid and new capsid protein.
- **Release** of virus progeny from the cell.

As shown on p. 376, viruses replicate only in living host cells. The detailed steps involved in their replication are shown below (Fig. 7.**4**). The reactions of the infected cell (cytopathology, tumor transformation, etc.) are described on p. 392.

Virus Replication

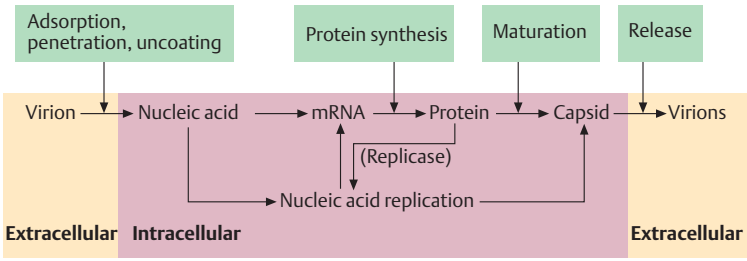
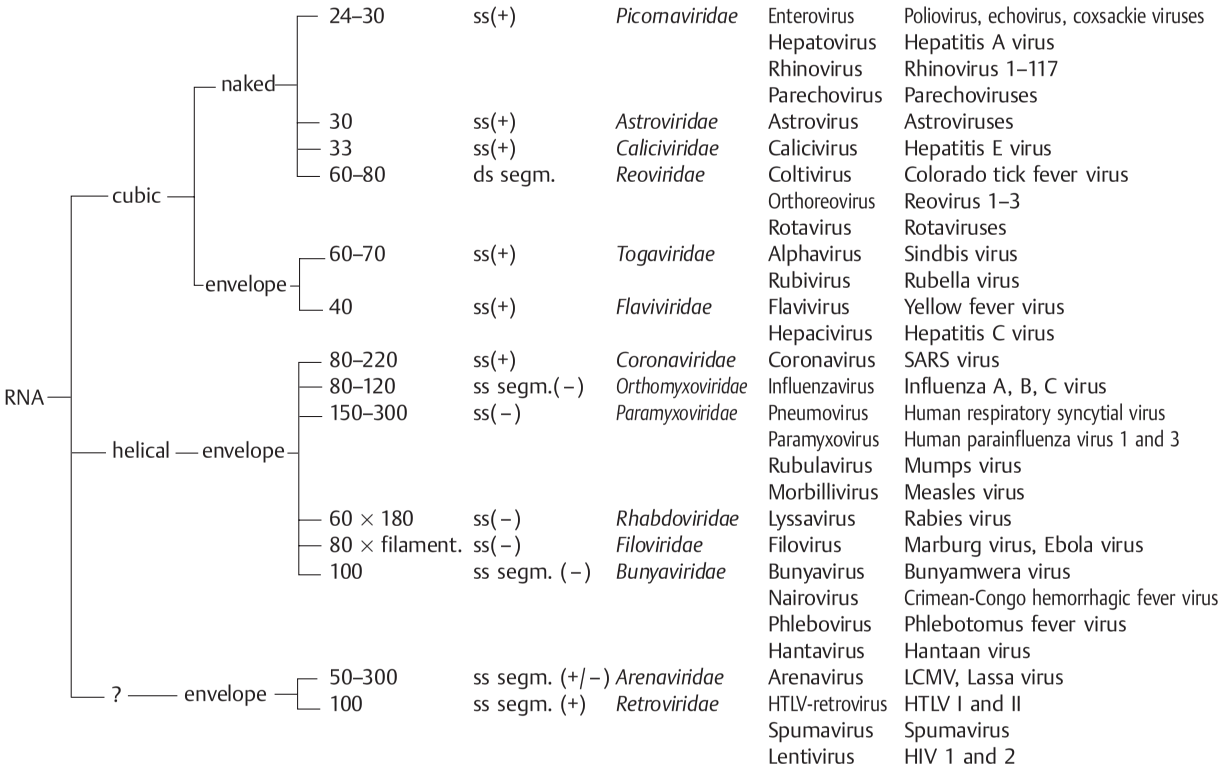


Fig. 7.**4** See text for details of each step.

Table 7.2 Taxonomy of the Viruses

Nucleic acid	Nucleo-capsid symmetry	Envelope	Virus diameter (nm)	ss/ds [1] (polarity)	Family	Genus	Exemplary important species
DNA	cubic	naked	19–25	ss	*Parvoviridae*	Erythrovirus	Parvovirus B19
			55	ds	*Papillomaviridae*	Papillomavirus	Human papilloma virus (HPV)
			45	ds	*Polyomaviridae*	Polyomavirus	BK virus, JC virus
			70–90	ds	*Adenoviridae*	Mastadenovirus	Adenoviruses
		envelope	27/42[2]	ss	*Hepadnaviridae*	Ortho-hepadnavirus	Hepatitis B virus
			100/200[2]	ds	*Herpesviridae*	Simplexvirus	Herpes simplex virus
						Varicellovirus	Varicella zoster virus
						Cytomegalo-virus	Cytomegalovirus
						Roseolovirus	Human herpesvirus 6
						Lymphocrypto-virus	Epstein-Barr virus
	complex	envelope	230 × 350	ds	*Poxviridae*	Orthopox	Variola virus, vaccinia virus
						Parapox	Orf virus



24–30	ss(+)	*Picornaviridae*	Enterovirus	Poliovirus, echovirus, coxsackie viruses
			Hepatovirus	Hepatitis A virus
			Rhinovirus	Rhinovirus 1–117
			Parechovirus	Parechoviruses
30	ss(+)	*Astroviridae*	Astrovirus	Astroviruses
33	ss(+)	*Caliciviridae*	Calicivirus	Hepatitis E virus
60–80	ds segm.	*Reoviridae*	Coltivirus	Colorado tick fever virus
			Orthoreovirus	Reovirus 1–3
			Rotavirus	Rotaviruses
60–70	ss(+)	*Togaviridae*	Alphavirus	Sindbis virus
			Rubivirus	Rubella virus
40	ss(+)	*Flaviviridae*	Flavivirus	Yellow fever virus
			Hepacivirus	Hepatitis C virus
80–220	ss(+)	*Coronaviridae*	Coronavirus	SARS virus
80–120	ss segm.(–)	*Orthomyxoviridae*	Influenzavirus	Influenza A, B, C virus
150–300	ss(–)	*Paramyxoviridae*	Pneumovirus	Human respiratory syncytial virus
			Paramyxovirus	Human parainfluenza virus 1 and 3
			Rubulavirus	Mumps virus
			Morbillivirus	Measles virus
60 × 180	ss(–)	*Rhabdoviridae*	Lyssavirus	Rabies virus
80 × filament.	ss(–)	*Filoviridae*	Filovirus	Marburg virus, Ebola virus
100	ss segm. (–)	*Bunyaviridae*	Bunyavirus	Bunyamwera virus
			Nairovirus	Crimean-Congo hemorrhagic fever virus
			Phlebovirus	Phlebotomus fever virus
			Hantavirus	Hantaan virus
50–300	ss segm. (+/–)	*Arenaviridae*	Arenavirus	LCMV, Lassa virus
100	ss segm. (+)	*Retroviridae*	HTLV-retrovirus	HTLV I and II
			Spumavirus	Spumavirus
			Lentivirus	HIV 1 and 2

[1] = Configuration of nucleic acid: ss = single-stranded, ds = double-stranded; [2] = without/with envelope

Adsorption. Virus particles can only infect cells possessing surface "receptors" specific to the particular virus species. When a virus encounters such a cell, it adsorbs to it either with the capsid or, in enveloped viruses, by means of envelope proteins. It is therefore the receptors on a cell that determine whether it can be infected by a certain virus.

Receptors

Some aspects of the nature of the receptors are known. These are molecules that play important roles in the life of the cell or intercellular communication, e.g., molecules of the immunoglobulin superfamily (CD4: receptor for HIV; ICAM-1: receptor for rhinoviruses), the complement (C3) receptor that is also the receptor for the Epstein-Barr virus, or glycoproteins the cellular functions of which are not yet known.

Practical consequences arise from this growing knowledge about the receptors: on the one hand, it aids in the development of antiviral therapeutics designed to inhibit the adsorption of the viruses to their target cells. On the other hand, the genetic information that codes for certain receptors can be implanted into cells or experimental animals, rendering them susceptible to viruses to which they would normally be resistant. An example of this application is the use in experimental studies of transgenic mice rendered susceptible to polioviruses instead of primates (e.g., on vaccine testing).

Penetration and uncoating. Viruses adsorbed to the cell surface receptors then penetrate into the cell by means of pinocytosis (a process also known as viropexis). In enveloped viruses, the envelope may also fuse with the cell membrane, releasing the virus into the cytoplasm. Adsorption of such an enveloped virus to two cells at the same time may result in cell fusion. The next step, known as uncoating, involves the release of the nucleic acid from the capsid and is apparently (except in the smallpox virus) activated by cellular enzymes, possibly with a contribution from cell membranes as well. The exact mechanism, which would have to include preservation of the nucleic acid in toto, is not known for all viruses.

Replication of the nucleic acid. Different processes are observed corresponding to the types and configurations of the viral genome (Fig. 7.**5**).

- **DNA viruses:** the replication of viral DNA takes place in the cell nucleus (exception: poxviruses). Some viruses (e.g., herpesviruses) possess replicases of their own. The smaller DNA viruses (e.g., polyomaviruses), which do not carry information for their own DNA polymerase, code for polypeptides that modify the cellular polymerases in such a way that mainly viral DNA sequences are replicated.

***Hepadnaviruses*:** the genome consists of an ssDNA antisense strand and a short sense strand (Fig. 7.**5e**). The infected cell transcribes an RNA sense strand ("template strand") from the antisense strand. This template strand is integrated in virus capsids together with an RT DNA polymerase. The polymerase synthesizes a complementary antisense DNA and, to "seal off" the ends of the genome, a short sense DNA from the template strand.

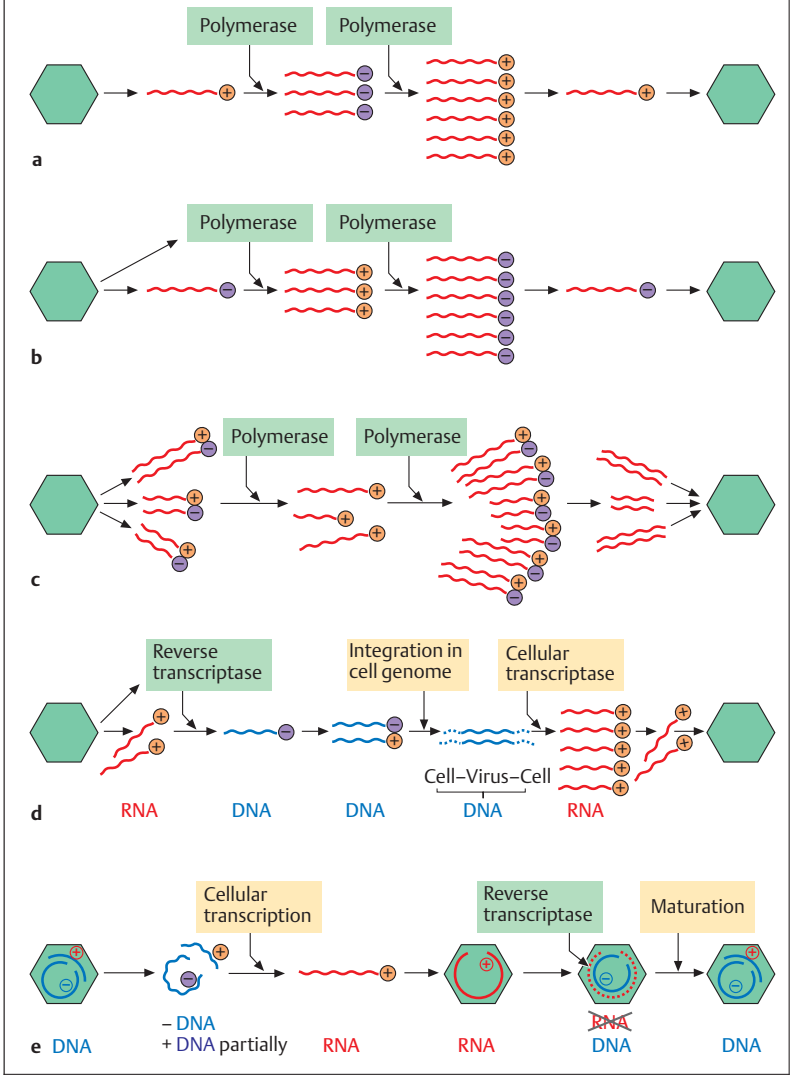
– **RNA viruses:** since eukaryotic cells possess no enzymes for RNA replication, the virus must supply the RNA-dependent RNA polymerase(s) ("replicase"). These enzymes are thus in any case virus-coded proteins, and in some cases are actually components of the virus particle.

***Single-stranded RNA*:** in *sense-strand viruses*, the RNA functions as mRNA "as is," meaning the information can be read off, and the replicase synthesized immediately. *Antisense-strand viruses* must first transcribe their genome into a complementary strand that can then act as mRNA. In this case, the polymerase for the first transcription is contained in the mature virion and delivered into the cell. In ssRNA viruses, whether sense or antisense strands, complementary strands of the genome are produced first (Fig. 7.5**a, b**), then transcribed into daughter strands. They therefore once again show the same polarity as the viral genome and are used in assembly of the new viral progeny.

***Double-stranded RNA*:** a translatable sense-strand RNA is produced from the genome, which consists of several dsRNA segments (segmented genome). This strand functions, at first, as mRNA and later as a matrix for synthesis of antisense-strand RNA (Fig. 7.**5c**). Here as well, an RNA-dependent RNA polymerase is part of the virus particle.

Retroviruses also possess a sense-oriented RNA genome, although its replication differs from that of other RNA viruses. The genome consists of two single-stranded RNA segments with sense polarity and is transcribed by an enzyme in the virion (reverse transcriptase [RT]) into complementary DNA. The DNA is complemented to make dsDNA and integrated in the cell genome. Transcription into sense-strand RNA is the basis for both viral mRNA and the genomic RNA in the viral progeny (Fig. 7.**5d**).

Replication of the Viral Genome



Viral Protein Synthesis

Production of viral mRNA. In a DNA virus infection, cellular polymerases transcribe mRNA in the nucleus of the host cell from one or both DNA strands, whereby the RNA is processed (splicing, polyadenylation, etc.) as with cellular mRNA. An exception to this procedure is the poxviruses, which use their own enzymes to replicate in the cytoplasm.

In *viruses with antisense-strand ssRNA and dsRNA* the transcription of the genomic RNA into mRNA is carried out by the viral polymerases, usually without further processing of the transcript.

In *sense-strand ssRNA viruses*, the genome can function directly as mRNA.

Certain viruses (arenaviruses, see p. 462f.) are classified as "*ambisense viruses*." Part of their genome codes in antisense (–), another part in sense (+) polarity. Proteins are translated separately from subgenomic RNA and the antisense-coded proteins are not translated until the antisense strand has been translated into a sense strand.

Viral mRNA is produced for the **translation** process, based on both the genome of the invading virus and the nucleic acid already replicated.

◀ Fig. 7.**5** Schematic diagram of nucleic acid replication.
a Single-stranded RNA viruses with sense-strand genome: the virus-coded RNA polymerase transcribes the viral genome (+) into complementary strands (–) and these into new genomic RNA (+). The latter is then integrated in the viral progeny.
b Single-stranded RNA viruses with antisense-strand genome: the RNA polymerase in the virion transcribes the viral genome (–) into complementary strands (+), which a virus-coded polymerase then transcribes into new genomic RNA (–).
c Double-stranded RNA viruses: while still in the partially decapsidated virus particle, the virus-coded polymerase transcribes complementary strands (+) from the antisense strand of the (segmented) double-stranded viral genome; these complementary strands are complemented to make the new double-stranded viral genome.
d RNA replication in retroviruses: the reverse transcriptase (RT) carried by the virion transcribes the viral genome (two sense-RNA strands) into complementary DNA (–), which is complemented to produce dsDNA and integrated in the cell genome. The viral RNA is first degraded. Cellular enzymes produce new genomic RNA (+).
e DNA replication in hepadnaviruses: by means of cellular transcription, a sense-strand RNA is made from the viral genome (antisense DNA, partially double-stranded) and integrated in the new virion, where a virus-coded RT produces new genomic DNA (–) and destroys the RNA.

The actual protein synthesis procedure is implemented, coded by the viral mRNA, with the help of cellular components such as tRNA, ribosomes, initiation factors, etc. Two functionally different protein types occur in viruses:

- The "noncapsid viral proteins" (NCVP) that do not contribute to capsid assembly. These proteins frequently possess enzymatic properties (polymerases, proteases) and must therefore be produced early on in the replication cycle.
- The capsid proteins, also known as viral proteins (VP) or structural proteins, appear later in the replication process.

Protein Synthesis Control

Segmented genomes. A separate nucleic acid segment is present for each protein (example: reoviruses).

mRNA splicing. The correct mRNA is cut out of the primary transcript (as in the cell the exon is cut out of the hnRNA) (examples: adenoviruses, retroviruses, etc.).

"Early" and "late" translation. The different mRNA molecules required for assembly of so-called early and late proteins are produced at different times in the infection cycle, possibly from different strands of viral DNA (examples, papovaviruses, herpesviruses).

Posttranslational control. This process involves proteolytic cutting of the primary translation product into functional subunits. Viral proteases that recognize specific amino acid sequences are responsible for this, e.g., the two poliovirus proteases cut between glutamine and glycine or tyrosine and glycine. Such proteases, some of which have been documented in radiocrystallographic images, are potential targets for antiviral chemotherapeutics (example: HIV).

Viral maturation (morphogenesis). In this step, the viral capsid proteins and genomes (present in multiple copies after the replication process) are assembled into new, infectious virus particles. In some viral species these particles are also covered by an envelope (p. 378f.)

Release. The release of viral progeny in some cases correlates closely with viral maturation, whereby envelopes or components of them are acquired when the particles "bud off" of the cytoplasmic membrane and are expelled from the cell (Fig. 7.**6**). In nonenveloped viruses, release of viral progeny is realized either by means of lysis of the infected cell or more or less continuous exocytosis of the viral particles.

Release of Retroviruses from an Infected Cell

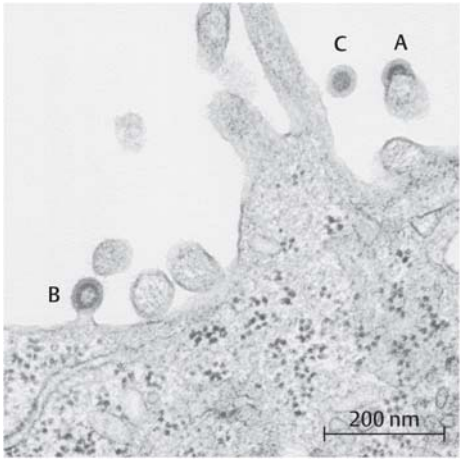


Fig. 7.**6** Electron microscope image of release of viral progeny. The process takes place in the order, A, B, C.

Genetics

Just as in higher life forms, viral genetic material is subject to change by mutation. Lack of a corrective replication "proofreading" mechanism results in a very high incidence of spontaneous mutations in RNA viruses, in turn greatly increasing the genotypic variability within each species ("viral quasispecies"). Furthermore, a potential for recombination of genetic material is also inherent in the replication process, not only material from different viruses but also from host cell and virus. This factor plays a major role in viral tumor induction and genetic engineering. Functional modifications arising from interactions between different viral species in mixed infections—e.g., phenotype mixing, interference, and complementation—have nothing to do with genetic changes.

Lasting genetic changes in viruses are caused, as in the higher life forms, either by mutation or recombination of genetic material. Temporary nongenetic interactions between viruses in some cases may mimic genetic changes.

Mutation. Mutations are changes in the base sequence of a nucleic acid, resulting in a more or less radical alteration of the resulting protein. So-called "silent mutations" (in the second or third nucleotide of a codon) do not influence the amino acid sequence of the protein.

Medically important are mutants with weakened virulence that have retained their antigenicity and replication capabilities intact. These are known as "attenuated" viruses. They are the raw material of live vaccines.

Recombination. The viral replication process includes production of a large number of copies of the viral nucleic acid. In cases where two different viral strains are replicating in the same cell, there is a chance that strand breakage and reunion will lead to new combinations of nucleic acid segments or exchanges of genome segments (influenza), so that the genetic material is redistributed among the viral strains (recombination). New genetic properties will therefore be conferred upon some of the resulting viral progeny, some of which will also show stable heritability. Genetic material can also be exchanged between virus and host cell by the same mechanism or by insertion of all, or part, of the viral genome into the cell genome.

Viruses as Vectors

The natural processes of gene transfer between viruses and their host cells described above can be exploited to give certain cells new characteristics by using the viruses as vectors. If the vector DNA carrying the desired additional gene integrates stably in the host cell genome (e.g., retroviruses, adenoviruses, or the adenoassociated virus), the host cell is permanently changed. This can become the basis for "gene therapy" of certain functional disorders such as cystic fibrosis or parkinsonism. Nonintegrating vectors (alphaviruses, e.g., the Sindbis virus, mengovirus, or vaccinia virus) result in temporary expression of a certain protein, which can be used, for instance, to immunize a host organism. By this means, wild foxes can be vaccinated against rabies using a vaccinia virus that expresses a rabies virus glycoprotein. Such experimental work must of course always comply with national laws on the release of genetically engineered microorganisms. It must also be mentioned here that only somatic gene therapy can be considered for use in humans. Human germline therapy using the methods of genetic engineering is generally rejected as unethical.

Nongenetic Interactions

In mixed infections by two (or more) viruses, various viral components can be exchanged or they may complement (or interfere with) each other's functions (phenotype mixing, complementation or interference). Such processes do not result in stable heritability of new characteristics.

In **phenotypic mixing**, the genome of virus A is integrated in the capsid of virus B, or a capsid made up of components from two (closely related) virus types is assembled and the genome of one of the "parents" is integrated in it. However, the progeny of such a "mixed" virus of course shows the genotype.

In phenotypic **interference**, the primary infecting virus (usually avirulent) may inhibit the replication of a second virus, or the inhibition may be mutual. The interference mechanism may be due to interferon production (p. 400) or to a metabolic change in the host cell.

In **complementation**, infecting viral species have genetic defects that render replication impossible. The "partner" virus compensates for the defect, supplying the missing substances or functions in a so-called helper effect. In this way, a defective and nondefective virus, or two defective viruses, can complement each other. Example: murine sarcoma viruses for which leukemia virus helpers deliver capsid proteins or the hepatitis D virus, which replicates on its own but must be supplied with capsid material by the hepatitis B virus (see Chapter 8, p. 429f.).

"Quasispecies." When viral RNA replicates, there is no "proofreading" mechanism to check for copying errors as in DNA replication. The result is that the rate of mutations in RNA viruses is about 10^4, i.e., every copy of a viral RNA comprising 10 000 nucleotides will include on average one mutation. The consequence of this is that, given the high rate of viral replication, all of the possible viable mutants of a viral species will occur and exist together in an inhomogeneous population known as quasispecies. The selective pressure (e.g., host immune system efficiency) will act to select the "fittest" viruses at any given time. This explains the high level of variability seen in HIV as well as the phenomenon that a single passage of the attenuated polio vaccine virus through a human vaccine recipient produces neurovirulent revertants.

Occurrence of "new" viral species. It appears to be the exception rather than the rule that a harmless or solely zoopathic virus mutates to become an aggressive human pathogen. In far more cases, changed environmental conditions are responsible for new forms of a disease, since most "new" viruses are actually "old" viruses that had reached an ecological balance with their hosts and then entered new transmission cycles as a result of urbanization, migra-

tion, travel, and human incursion into isolated biotopes (examples include the Ebola, Rift Valley fever, West Nile, pulmonary Hanta, and bat rabies viruses).

Host-Cell Reactions

- Possible consequences of viral infection for the host cell:
- **Cytocidal infection (necrosis):** viral replication results directly in cell destruction (cytopathology, so-called "cytopathic effect" in cell cultures).
- **Apoptosis:** the virus initiates a cascade of cellular events leading to cell death ("suicide"), in most cases interrupting the viral replication cycle.
- **Noncytocidal infection:** viral replication per se does not destroy the host cell, although it may be destroyed by secondary immunological reactions.
- **Latent infection:** the viral genome is inside the cell, resulting in neither viral replication nor cell destruction.
- **Tumor transformation:** the viral infection transforms the host cell into a cancer cell, whereby viral replication may or may not take place depending on the virus and/or cell type involved.

Cell Destruction (Cytocidal Infection, Necrosis)

Cell death occurs eventually after initial infection with many viral species. This cytopathological cell destruction usually involves production of viral progeny. Virus production coupled with cell destruction is termed the "lytic viral life cycle." Cell destruction, whether necrotic or apoptotic (see below) is the reason (along with immunological phenomena) for the disease manifested in the macroorganism (see Pathogenesis, p. 396ff.).

Structural changes leading to necrosis: morphological changes characteristic of a given infecting virus can often be observed in the infected cell. The effects seen in virally infected cell cultures are well-known and are designated by the term "cytopathic effect" (CPE). These effects can also be exploited for diagnostic purposes (p. 405). They include rounding off and detachment of cells from adjacent cells or the substrate, formation of multinuclear giant cells, cytoplasmic vacuoles, and inclusion bodies. The latter are structures made up of viral and/or cellular material that form during the viral replication cycle, e.g., viral crystals in the nucleus (adenoviruses) or collections of virions and viral material in the cytoplasm (smallpox viruses). Although these structural changes in the host cell do contribute

to necrotic cytopathy, their primary purpose is to support specific steps in viral synthesis. For example, RNA synthesis and viral assembly in picornavirus infections requires specific, new, virus-induced membrane structures and vesicles that subsequently manifest their secondary effect by causing a CPE and eventual cell death.

Shutoff Phenomena

Some viruses are able to block, more or less completely, steps in cellular macromolecule synthesis not useful to them. Herpesviruses, for example, which possess DNA polymerase of their own, block cellular DNA synthesis. DNA replication in adenoviruses, by contrast, is directly coupled to that of the cell. Such shutoff phenomena apparently contribute to rapid and efficient viral replication by eliminating competing cellular synthetic processes. In polioviruses, which inhibit both transcription and translation in the host cell, the shutoff processes are induced by viral proteins that interfere with the relevant regulatory mechanisms in order to inhibit transcription and to inactivate initiation factor eIF4GII, which is not required for translation of *Enterovirus* mRNA, in order to inhibit translation. These shutoff phenomena of course also have a pathogenic effect since they inhibit cellular metabolism, but not in such a way as to necessarily kill the host cell.

Apoptosis. Cells possess natural mechanisms that initiate their self-destruction (apoptosis) by means of predetermined cytoplasmic and nuclear changes. Infections with some viruses may lead to apoptosis. In rapidly replicating viruses, the viral replication process must be decelerated to allow the slow, energy-dependent process of apoptosis to run its course before the cell is destroyed by virus-induced necrosis. The body rapidly eliminates apoptotic cells before an inflammatory reaction can develop, which is apparently why virus-induced apoptosis used to be overlooked so often. Apoptosis can thus be considered a defense mechanism, although certain viruses are able to inhibit it.

Virus Replication without Cell Destruction (Noncytocidal Infection)

This outcome of infection is observed with certain viruses that do not cause any extensive restructuring of the host cell and are generally released by "budding" at the cell surface. This mode of replication is seen, for example, in the oncornaviruses and myxoviruses and in the chronic form of hepatitis B virus infection. However, cell destruction can follow as a secondary result of infection, however, if the immune system recognizes viral antigens on the cell surface, classifies it as "foreign" and destroys it.

Latent Infection

In this infection type, the virus (or its genome) is integrated in a cell, but no viral progenies are produced. The cell is accordingly not damaged and the macroorganism does not manifest disease. This form of infection is found, for instance, with the adenovirus group and in particular the herpesviruses, which can remain latent for long periods in the human body. Latency protects these viruses from immune system activity and thus is part of their survival strategy. However, a variety of initiating events (see Chapter 8, p. 419) can initiate a lytic cycle leading to manifest disease and dissemination of the virus. Repeated activation of a latent virus is termed recidivation (e.g., herpes labialis).

Tumor Transformation

Infections by a number of viruses do not result in eventual host cell death, but rather cause tumor transformation of the cell. This means the cell is altered in many ways, e.g., in its growth properties, morphology, and metabolism. Following an infection with DNA tumor viruses, the type of host cell infected determines whether the cell reaction will be a tumor transformation, viral replication or lytic cycle. The transformation that takes place after infection with an RNA tumor virus either involves no viral replication (nonpermissive infection) or the cell produces new viruses but remains vital (permissive infection).

Carcinogenic Retroviruses ("Oncoviruses")

Genome structure and replication of the oncoviruses. The genomes of all oncoviruses possess *gag* (group-specific antigen), *pol* (enzymatic activities: polymerase complex with reverse transcriptase, integrase, and protease), and *env* (envelope glycoproteins) genes. These coding regions are flanked by two control sequences important for regulatory functions called LTR (= long terminal repeats), Fig. 7.**7**. These sequences have a promoter/enhancer function and are responsible for both reverse transcription and insertion of the viral genome into the cell DNA. Certain oncoviruses possess a so-called "*onc* gene" instead of the *pol* region (*onc* gene = oncogene, refers to a cellular gene segment acquired by recombination, see below). These viruses also often have incomplete *gag* and/or *env* regions. Such viruses are defective and require a helper virus to replicate (complementation, see p. 391). An exception to this principle is the Rous sarcoma virus, which possesses both an *onc* gene and a complete set of viral genes and can therefore replicate itself.

Genomic Organization in Oncoviruses

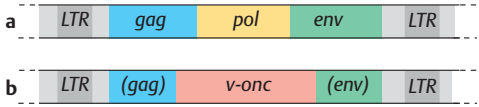


Fig. 7.7 **a** Autonomously reproducing oncoviruses with the three replication genes *gag*, *pol*, *env*, flanked by the LTR regions.
b Defective oncoviruses contain an *onc* gene instead of the entire *pol* region and parts of the *gag* and *env* regions.

Oncogenes

Over 100 *onc* genes (so-called "oncogenes") have been found in the course of tumor virology research to date. These genes enable tumor viruses to transform their host cells into tumor cells. The various types of oncogenes are designated by abbreviations, in most cases derived from the animal species in which the virus was first isolated. Further investigation of these viral oncogenes have now shown that these genes are not primarily of viral origin, but are rather **normal, cellular genes** widespread in humans and animals and acquired by the oncoviruses in their host cells, which can be transferred to new cells (transduction). Such a cellular gene, not oncogenic per se, is called a **proto-oncogene**.

The normal function of the proto-oncogenes concerns the regulation of cell growth in the broadest sense. Their gene products are growth factors, growth factor or hormone receptors and GTP-binding or DNA-binding proteins. Proto-oncogenes are potential contributors to tumor development that have to be **"activated"** before they can actually have such effects. This can occur by way of several different mechanisms:

- Chromosomal translocation: proto-oncogenes are moved to different chromosomes and thus placed under the influence of different cellular promoters, resulting in a chronic overexpression of the corresponding protein.
- Mutation of the proto-oncogene.
- Transduction of the proto-oncogene by an oncovirus. The oncovirus promoter may induce overexpression of the proto-oncogene, resulting in a tumor.

Tumor induction by oncoviruses. Both types of carcinogenic retroviruses, i.e., those with no oncogene and intact replication genes (*gag*, *pol*, *env*, flanked by the LTR regions) and those that have become defective by taking on an oncogene, can initiate a tumor transformation. On the whole, oncoviruses play only a subordinate role in human tumor induction.

Retroviruses without an oncogene: LTR are highly effective promoters. Since the retrovirus genome is integrated in the cell genome at a random

position, the LTR can also induce heightened expressivity in cellular proto-oncogenes ("promoter insertion hypothesis" or "insertion mutagenicity"), which can lead to the formation of tumors. This is a slow process (e.g., chronic leukemias) in which cocarcinogens can play an important role. The transformed cells produce new viruses.

■ **Retroviruses with an oncogene:** a viral oncogene always represents a changed state compared with the original cellular proto-oncogene (deletion, mutation). It is integrated in the cell genome together with the residual viral genome (parts) after reverse transcription, and then expressed under the influence of the LTR, in most cases overexpressed. This leads to rapid development of acute malignancies that produce no new viruses.

Overproduction of oncogene products can be compensated by gene products from antioncogenes. The loss or mutation of such a suppressor gene can therefore result in tumor formation.

DNA Tumor Viruses

Genes have also been found in DNA tumor viruses that induce a malignant transformation of the host cell. In contrast to the oncogenes in oncoviruses, these are genuine viral genes that have presumably developed independently of one another over a much longer evolutionary period. They code for viral regulator proteins, which are among the so-called early proteins. They are produced early in the viral replication cycle and assume essential functions in viral DNA replication. Their oncogenic potential derives among other things from the fact that they bind to the products of tumor suppressor genes such as *p53*, *Rb* (antioncogenes, "antitransformation proteins" see above) and can thus inhibit their functions. DNA viruses are more important inducers of human tumors than oncoviruses (example: HHV8, papovaviruses, hepatitis B viruses, Epstein-Barr viruses).

Pathogenesis

■ The term "pathogenesis" covers the factors that contribute to the origins and development of a disease. In the case of viruses, the infection is by a parenteral or mucosal route. The viruses either replicate at the portal of entry only (**local infection**) or reach their target organ hematogenously, lympogenously or by neurogenic spread (**generalized infection**). In both cases, viral replication induces degenerative damage. Its extent is determined by the extent of virus-induced cell destruction and sets the level of disease mani-

festation. Immunological responses can contribute to elimination of the viruses by destroying the infected cells, but the same response may also exacerbate the course of the disease.

Transmission. Viruses can be transmitted horizontally (within a group of individuals (Table 7.**3**) or vertically (from mother to offspring). Vertical infection is either transovarial or by infection of the virus in utero (ascending or diaplacental). Connatal infection is the term used when offspring are born infected.

Portal of entry. The most important portals of entry for viruses are the mucosa of the respiratory and gastrointestinal tracts. Intact epidermis presents a barrier to viruses, which can, however, be overcome through microtraumata (nearly always present) or mechanical inoculation (e.g., bloodsucking arthropods).

Viral dissemination in the organism. There are two forms of infection:

- **Local infection.** In this form of infection, the viruses spread only from cell to cell. The infection and manifest disease are thus restricted to the tissues in the immediate vicinity of the portal of entry. Example: rhinoviruses that reproduce only in the cells of the upper respiratory tract.

- **Generalized infection.** In this type, the viruses usually replicate to some extent at the portal of entry and are then disseminated via the *lymph ducts* or *bloodstream* and reach their target organ either directly or after infecting a further organ. When the target organ is reached, viral replication and the resulting cell destruction become so widespread that clinical symptoms develop. Examples of such infection courses are seen with enteroviruses that replicate mainly in the intestinal epithelium, but cause no symptoms there.

Table 7.**3** Horizontal Transmission of Pathogenic Viruses

Mode of transmission	Examples
Direct transmission	
– fecal-oral (smear infection)	Enteroviruses
– aerogenic (droplet infection)	Influenza viruses
– intimate contact (mucosa)	Herpes simplex virus
Indirect transmission	
– alimentary	Hepatitis A virus
– arthropod vectors	Yellow fever virus
– parenteral	Hepatitis B virus

Clinical symptoms in these infections first arise in the target organs such as the CNS (polioviruses, echoviruses) or musculature (coxsackie viruses).

Another mode of viral dissemination in the macroorganism is neurogenic spread along the *nerve tracts*, from the portal of entry to the CNS (rabies), or in the opposite direction from the ganglions where the viruses persist in a latent state to the target organ (herpes simplex).

Organ Infections, Organotropism

Whether a given cell type can be infected by a given viral species at all depends on the presence of certain receptors on the cell surface (p. 384). This mechanism explains why organotropism is observed in viruses. However, the tropism is only apparent; it is more accurate to speak of susceptible and resistant cells (and hence organs). Another observation is that cells grown in the laboratory in cell cultures can completely change their sensitivity or resistance to certain viral species compared with their organ of origin.

Course of infection. The organ damage caused by viruses is mainly of a degenerative nature. Inflammatory reactions are secondary processes. The severity of the clinical symptoms depends primarily on the extent of virus-induced (or immunological, see below) cell damage. This means most of the viral progeny are produced prior to the occurrence of clinical symptoms, with consequences for epidemiology and antiviral therapies (p. 404). It also means that infections can go unnoticed if cell destruction is insignificant or lacking entirely. In such cases, the terms *inapparent*, silent, or subclinical infection are used, in contrast to *apparent* viral infections with clinical symptoms. Virus replication and release do take place in inapparent infections, as opposed to *latent* infections (p. 394), in which no viral particles are produced.

Immunological processes can also influence the course of viral infections, whereby the infection can be subdued or healed (p. 401ff.). On the other hand, the infection may also be exacerbated, either because immune complexes are formed with viruses or viral components (nephritis) or because the immune system recognizes and destroys virus-infected cells. This is possible if viral antigens are integrated in the cell membrane and thus expressed on the cell surface. These processes become pathologically significant in cases in which the viruses themselves cause little or no cell destruction (p. 393).

Antibody-Dependent Enhancement of Viral Infection

The disease process can also be worsened when viruses react with subneutralizing amounts or types of antibodies. The Fc fragment of the antibodies bound to the viruses can then react with the Fc receptors on specific cells. This makes it possible for cell types to be infected that are primarily resistant to the virus in question because they possess no viral receptors (but in any case Fc receptors). This process—called "antibody-dependent enhancement of viral infection" or ADE, reflecting the fact that the antibodies exacerbate the infection—has been experimentally confirmed with a number of virus types to date, including herpes virus, poxvirus, reovirus, flavivirus, rhabdovirus, coronavirus, bunyavirus, and HIV species.

Virus excretion. Excretion of newly produced viruses depends on the localization of viral replication. For example, viruses that infect the respiratory tract are excreted in expired air (droplet infection). It must be remembered that in generalized infections not only the target organ is involved in excretion, but that primary viral replication at the portal of entry also contributes to virus excretion (for example enteroviruses, which replicate primarily in the intestinal wall and are excreted in feces). Once again, since the symptoms of a viral disease result from cell destruction, production, and excretion of new virus progeny precede the onset of illness. As a rule, patients are therefore contagious before they really become ill.

Defense Mechanisms

The mechanisms available to the human organism for defense against viral infection can be classified in two groups. The **nonspecific immune defenses**, in which interferons play a very important part, come first. Besides their effects on cell growth, immune response, and immunoregulation, these substances can build up a temporary resistance to a viral infection. Interferons do not affect viruses directly, but rather induce cellular resistance mechanisms (synthesis of "antiviral proteins") that interfere with specific steps in viral replication. The **specific immune defenses** include the *humoral immune system*, consisting mainly of antibodies, and the *cellular immune system*, represented mainly by the T lymphocytes. In most cases, cellular immunity is more important than humoral immunity. The cellular system is capable of recognizing and destroying virus-infected cells on the surfaces of which viral antigens are expressed. The humoral system can eliminate only extracellular viruses.

Nonspecific Immune Defenses

The nonspecific immune defense mechanisms are activated immediately when pathogens penetrate the body's outer barriers. One of the most important processes in these basic defenses is phagocytosis, i.e., ingestion and destruction of pathogens. Granulocytes and natural killer cells bear most of the responsibility in these mechanisms. Changes in pH and ion balance as well as fever also play a role, for example, certain temperature-sensitive replication steps can be blocked. The most important humoral factor is the complement system. Interferons, which are described below, are also potent tools for fighting off viral infections. The other mechanisms of nonspecific immune defense are described in Chapter 2, (Principles of Immunology, p. 43ff.).

Interferons (IFN) are cell-coded proteins with a molecular weight of about 20 kDa. Three types are differentiated (leukocyte interferon = IFNα, fibroblast interferon = IFNβ, and immune interferon = IFNγ) of which the amino acid sequences are known and which, thanks to genetic engineering, can now be produced in practically unlimited amounts. Whereas the principal biological effects of interferons on both normal and malignant cells are antiviral and antimitotic, these substances also show immunomodulatory effects. Their clinical applications are designed accordingly. In keeping with the scope of this section, the following description of their antiviral activity will be restricted to the salient virological aspects (Fig. 7.**8**):

A number of substances can induce the production of interferon in a cell, for example double-stranded RNA, synthetic or natural polynucleotides, bacteria, various low-molecular compounds and, above all, viruses. All of these

Synthesis and Effects of Interferon

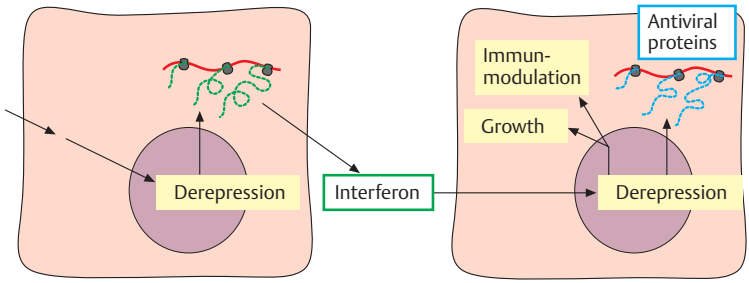


Fig. 7.**8** An interferon-inducing substance initiates production of interferon in the first cell. In the second cell, interferon induces, for instance, production of antiviral proteins.

substances have the same effect: they derepress the cellular *interferon* gene, inducing the cell to begin producing interferon precursors. Following glycosylation, the finished interferon is released into the surrounding area and binds to the interferon receptor of the nearest cell. The presence or lack of this receptor determines what effect the interferon will have. It also explains the more or less pronouncedly species-specific nature of the cell-to-interferon relationship. In principle, the effect of interferon is strongest within the species in which it was produced. Within the recipient or "target" cell the interferon induces the expression of the so-called *interferon-stimulated genes* (ISG) by means of a signal cascade, the result of which is to inhibit viral replication.

Interferon-Induced Proteins

(2'-5')(A)n synthetase. This cellular enzyme is first produced in an inactive form. It is then activated by double-stranded RNA, after which it can polymerize oligoadenylate out of ATP. This product then activates a cellular ribonuclease (RNase L), which inactivates viral (and cellular) mRNA.

P1/eIF-2 kinase. This cell-coded kinase is also inactive in its native state and must also be activated by dsRNA. It is then able to phosphorylate the ribosomal protein P1 and the initiation factor eIF-2, resulting in inhibition of protein synthesis initiation.

How viral and cellular protein synthesis are told apart in this kinase activation process is not quite clear. Perhaps the dsRNA needed to activate the enzyme is the key: this substance is lacking in noninfected cells and is only produced in cells infected by an (RNA) virus, so that the antiviral enzymes can only be activated in the infected cells.

Mx protein. The observation that certain mice are resistant to influenza viruses led to the discovery of the interferon-induced, 75–80 kDa Mx proteins coded for by dominant hereditary *Mx* genes. Mx proteins accumulate in mouse cell nuclei and inhibit the mRNA synthesis of influenza viruses. Mx^- mice are killed by influenza. In humans, Mx proteins accumulate in the cytoplasm, but their mechanism of action is unknown.

Specific Immune Defenses

The specific, adaptive immune defenses include both the humoral system (antibody-producing B cells) and the cellular system (T helper cells and cytotoxic T lymphocytes). In general, viruses the antigens of which are expressed on the surface of the infected cells tend to induce a cellular immune

response and viruses that do not change the antigenicity of their host cells tend to activate the humoral system.

Humoral immunity. Antibodies can only attack viruses outside of their host cells, which means that once an infection is established within an organ it can hardly be further influenced by antibodies, since the viruses spread directly from cell to cell. In principle, the humoral immune system is thus only capable of preventing a generalized infection, but only if the antibodies are present at an early stage (e.g., induced by a vaccination). Class IgG and IgM antibodies are active in the bloodstream (see Chapter 2) and class IgA is active on the mucosal surface. The effect of the antibodies on the viral particles ("neutralization") is based on steric hindrance of virus adsorption to the host cells by the antibodies attached to their surfaces. The neutralizing effect of antibodies is strongest when they react with the receptor-binding sites on the capsids so as to block them, rendering the virus incapable of combining with the cellular receptors (p. 384).

Cellular immunity. This type of immune defense is far more important when it comes to fighting viral infections. T lymphocytes (killer cells) recognize virus-infected cells by the viral antigens on their surfaces and destroy them. The observation that patients with defective humoral immunity generally fare better with virus infections than those with a defective cellular response underlines the fact that the cellular immune defense system is the more important of the two.

Prevention

■ The most important prophylactic measures in the face of potential viral infections are active vaccines. Vaccines containing inactivated viruses generally provide shorter-lived and weaker protection than live vaccines. Passive immunization with human immunoglobulin is only used in a small number of cases, usually as postexposure prophylaxis. ■

Value of the different methods. In general, vaccination, i.e., induction of immunity (*immune prophylaxis*) is the most important factor in prevention of viral infection. *Exposure prophylaxis* is only relevant to hygienic measures necessitated by an epidemic and is designed to prevent the spread of pathogens in specific situations. *Chemoprophylaxis*, i.e., administration of chemotherapeutic agents when an infection is expected instead of after it has been diagnosed to block viral metabolism, is now justified in selected cases, e.g., in immunosuppressed patients (see Chemotherapy, p. 404).

There are two basic types of vaccines:

Active immunization. In this method, the antigen (virus) is introduced into the body, either in an inactivated form, or with attenuated pathogenicity but still capable of replication, to enable the body to build up its own immunity.

- **Inactivated vaccines.** The immunity that develops after so-called "dead vaccines" are administered is merely humoral and generally does not last long. For this reason, booster vaccinations must be given repeatedly. The most important dead vaccines still in use today are influenza, rabies, some flavivirus, and hepatitis A and B vaccines. Some inactivated vaccines contain the most important immunogenic proteins of the virus. These so-called split vaccines induce more efficient protection and, above all, are better tolerated. Some of them are now produced by genetic engineering methods.

- **Live attenuated vaccines.** These vaccines confer effective and long-lasting protection after only a single dose, because the viruses contained in them are capable of replication in the body, inducing not only humoral, but sometimes cellular immunity as well, not to mention local immunity (portal of entry!). Such live vaccines are preferable when available. There are, however, also drawbacks and risks, among them stability, the increased potential for contamination with other viruses, resulting in more stringent testing and the possibility that a back-mutation could produce a pathogenic strain (see Variability and Quasispecies of Viruses, p. 391).

- **Vaccines with recombinant viruses.** Since only a small number of (surface) viral proteins are required to induce protective immunization, viral vectors are used in attempts to express them in vaccine recipients (see p. 390). Suitable vectors include the least virulent virus strains among the picornaviruses, alphaviruses, and poxviruses. There must be no generalized immunity to the vector in the population so that it can replicate in vaccine recipients and the desired protein will at the same time be expressed. Such recombinant vaccines have not yet been approved for use in humans. A rabies vaccine containing the recombinant vaccinia virus for use in animals is the only practical application of this type so far (p. 390).

- **Naked DNA vaccine.** Since pure DNA can be inserted into eukaryotic cells (transfection) and the information it carries can be expressed, DNA that codes for the desired (viral) proteins can be used as vaccine material. The advantages of such vaccines, now still in the trial phase, include ease of production and high stability.

Passive immunization. This type of vaccine involves the injection of antibodies using only human immunoglobulins. The protection conferred is of short duration and only effective against viruses that cause viremia. Passive immunization is usually administered as a postexposure prophylactic measure, i.e.,

after an infection or in situations involving a high risk of infection, e.g., to protect against hepatitis B and rabies (locally, bite wound). Table 1.**13** (p. 33) and Table 8.**7** (rabies, p. 470) list the most important vaccines.

Chemotherapy

■ Inhibitors of certain steps in viral replication can be used as chemotherapeutic agents to treat viral infections. In practical terms, it is much more important to inhibit the synthesis of viral nucleic acid than of viral proteins. The main obstacles involved are the low level of specificity of the agents in some cases (toxic effects because cellular metabolism is also affected) and the necessity of commencing therapy very early in the infection cycle. ■

Problems of chemotherapy. As described on p. 381, viral replication is completely integrated in cell metabolism. The virus supplies only the genetic in-

Table 7.**4** The Most Important Antiviral Chemotherapeutics

Chemotherapeutic agent	Effect/indication
Adamantanamin (amantadine)	Inhibition of uncoating in influenza viruses
Acycloguanosine (acyclovir, Zovirax)	Inhibition of DNA synthesis in HSV and VZV
Dihydropropoxymethylguanosine (DHPG, ganciclovir, Cymevene)	Inhibition of DNA synthesis in CMV
Ribavirin	Inhibition of mRNA synthesis and capping. Infections with Lassa virus and perhaps in severe paramyxovirus and myxovirus infections
Nucleoside RT inhibitors (NRTI)	Inhibition of RT in HIV (p. 454)
Phosphonoformate (foscarnet)	Inhibition of DNA synthesis in herpesviruses, HIV, HBV
Protease inhibitors	Inhibition of viral maturation in HIV
Neuraminidase inhibitors	Inhibition of release of influenza viruses
Antisense RNA	Complementary to viral mRNA, which it blocks by means of hybridization (duplexing)

formation for proteins to be synthesized by the cell. This close association between viruses and their host cells is a source of some essential difficulties encountered when developing virus-specific chemotherapeutics, since any interference with viral synthesis is likely to affect physiological cellular synthetic functions as well. Specific intervention is only possible with viruses that code for their own enzymes (e.g., polymerases or proteases), which enzymes also react with viral substrates. Another problematic aspect is the necessity of administering chemotherapeutics (Table 7.**4**) early, preferably before clinical symptoms manifest, since the peak of viral replication is then usually already past (p. 399).

Development of resistance to chemotherapeutics. Acyclovir-resistant strains of herpesviruses, in particular herpes simplex viruses, are occasionally isolated. Less frequently, cytomegaly viruses resistant to ganciclovir are also found. These viruses possess a thymidine kinase or DNA polymerase altered by mutation. Infections caused by resistant herpesviruses are also observed in immunodeficient patients; the pathogens no longer respond to therapy after long-term treatment of dermal or mucosal efflorescences.

There are, as yet, no standardized resistance tests for chemotherapy-resistant viruses, so that the usefulness of such test results is of questionable value in confirmed cases. Also, the results obtained in vitro unfortunately do not correlate well with the cases of resistant viruses observed in clinical settings.

Laboratory Diagnosis

■ The following methods can be used to obtain a virological laboratory diagnosis:

- **Virus isolation** by growing the pathogen in a compatible host; usually done in cell cultures, rarely in experimental animals or hen embryos.
- **Direct virus detection.** The methods of serology, molecular biology, and electron microscopy are used to identify viruses or virus components directly, i.e., without preculturing, in diagnostic specimens.
- **Serodiagnostics** involving assay of antiviral antibodies of the IgG or IgM classes in patient serum. ■

Indication and methods. Laboratory diagnostic procedures for virus infections are costly, time-consuming, and require considerable staff time. It is therefore important to consider carefully whether such tests are indicated in a confirmed case. The physician in charge of treatment must make this decision based on detailed considerations. In general, it can be said that

Table 7.**5** Virological Laboratory Diagnostics

Diagnostic approach	Methods	Detection/identification of	Advantages/disadvantages
Isolation	Growing in cell cultures	Infectivity, pathogenicity	Slow but sensitive method
Direct detection	Electron microscopy, EIA, IF, hybridization, PCR	Viral particles, antigens, genome	Fast method, but may be less sensitive
Serology	EIA, IF, etc.	Antibodies	Retrospective method

laboratory diagnostics are justified if further treatment of the patient would be influenced by an etiological diagnosis or if accurate diagnostic information is required in the context of an epidemic or scientific research and studies.

There are essentially three different methods used in virological diagnostics (Table 7.**5**):

1. *Virus isolation* by growing the pathogen in a compatible host; usually done in cell cultures.
2. *Direct virus detection* in patient material; identification of viral particles using electron microscopy, viral antigens with the methods of serology, and viral genome (components) using the methods of molecular biology.
3. *Antibody assay* in patient serum.

General guidelines for viral diagnostics are listed below. Specific details on detection and identification of particular viral species are discussed in the relevant sections of Chapters 8 and 12.

Virus Isolation by Culturing

In this approach, the virus is identified based on its infectivity and pathogenicity by inoculating a host susceptible for the suspected virus—in most cases cell cultures—with the specimen material. Certain changes observed in the culture (cytopathic effect [CPE] p. 392f.) indicate the presence of a virus.

Cell Cultures

A great majority of viruses can be grown in the many types of human or animal cells available for culture. So-called primary cell cultures can be created with various fresh tissues. However, the cells in such primary cultures can only divide a limited number of times. Sometimes so-called cell lines can be developed from primary cultures with unlimited in-vitro culturing capacity. Well-known examples of this phenomenon are HeLa cells (human portio carcinoma cells) and Vero cells (monkey renal fibroblasts). For diagnostic purposes, the cell cultures are usually grown as "monolayers," i.e., a single-layer cell film adhering to a glass or plastic surface.

Viral replication in cell cultures results in morphological changes in the cells such as rounding off, formation of giant cells, and inclusion bodies (so-called CPE, see also p. 392f.). The CPE details will often suffice for an initial approximate identification of the virus involved.

Sampling and transport of diagnostic specimens. Selection of suitable material depends on the disease and suspected viral species (see Chapter 8). Sampling should generally be done as early as possible in the infection cycle since, as was mentioned on p. 399, viral replication precedes the clinical symptoms. Sufficiently large specimens must be taken under conditions that are as sterile as possible, since virus counts in the diagnostic material are almost always quite low. Transport must be arranged quickly and under cold box conditions. The half-life of viruses outside the body is often very short and must be extended by putting the material on ice. A number of virus transport mediums are commercially available. A particular transport medium should be selected after consulting the laboratory to make sure the medium is compatible with the laboratory methods employed. Such mediums are particularly important if the diagnostic material might otherwise dry out.

Information provided to the laboratory. The laboratory must be provided with sufficient information concerning the course and stage of the disease, etc. This is very important if the diagnostic procedure is to be efficient and the results accurate. Clinical data and tentative diagnoses must be provided so the relevant viruses can be looked for in the laboratory. Searching for every single virus potentially present in the diagnostic material is simply not feasible for reasons of cost and efficiency.

Laboratory processing of the material. Before the host is inoculated with the specimen material for culturing, contaminant bacteria must be eliminated with antibiotics, centrifugation, and sometimes filtering. All of these manipulations of course entail the risk of virus loss and reduction of test sensitivity, so the importance of sterile sampling cannot be overemphasized. In a few cases, virus enrichment is indicated, e.g., by means of ultracentrifugation.

Selection of a host system. The host system to be used is chosen based on the suspected (and relevant) virus infectors. Observation and incubation times, and thus how long a laboratory diagnosis will take, also depend on the viral species under investigation.

Identification of the viruses is based first on the observed cell changes, then determined serologically using known antibodies and appropriate methods such as immunoelectron microscopy, EIA, or the neutralization test (see p. 402 for the neutralization mechanism). Methods that detect the viral genome by means of in-situ or filter hybridization are now seeing increasing use.

Significance of results. The importance of virus isolation depends on the virus type. In most cases, isolation will be indicative of the etiology of the patient's disease. In some cases, (in particular the herpesvirus and adenovirus group, see Chapter 8), latent viruses may have been activated by a completely different disease. In such cases, they may of course be isolated, but have no causal connection with the observed illness.

Isolation is the most sensitive method of viral diagnostic detection, but it cannot detect all viruses in all situations. This means that a negative result does not entirely exclude a viral infection. Another aspect is that the methods of virus isolation, with few exceptions, detect only mature, infectious virions and not the latent viruses integrated in the cells. This renders diagnostic isolation useless during latency (e.g., herpes simplex between recidivations).

Amplification culture. In this method, the virus is grown for a brief period in a cell culture. Before the CPE is observed, the culture is tested using the antigen and genomic methods described. This is also known as a "shell vial assay" because the cells are grown on coverslips in shell vials (test tubes with screw caps). Using this arrangement, method sensitivity can be increased by centrifuging the diagnostic material onto the cell monolayer. The greatest amount of time is saved by detecting the virus-specific proteins produced early in the infection cycle, which is why the search concentrates on such so-called "early antigens" (see p. 388). Using this method, the time required to confirm a cytomegaly virus, for instance, can be shortened from four to six weeks to only two to five days with practically no loss of sensitivity compared to classic isolation methods.

Direct Virus Detection

In this diagnostic approach, the viruses are not identified as infectious units per se, but rather as viral particles or parts of them. The idea is to find the viruses directly in the patient material without prior culturing or replication. Viruses in serous fluids such as the contents of herpes simplex or varicella-

zoster blisters can be viewed under the electron microscope (EM). It must be remembered, however, that the EM is less sensitive than virus isolation in cultures by a factor of 10^5. Viral antigens can be detected in secretions using enzyme immunoassay (EIA), passive agglutination, or in smears with immunofluorescence performed with known antibodies, for instance monoclonal antibodies. Analogously, the viral genome can be identified by means of filter hybridization, or in smears or tissue sections with in-situ hybridization using DNA or RNA complementary to the viral genome as a probe.

Sampling and transport of diagnostic specimens. Transport of patient material for these methods is less critical than for virus isolation. Cold box transport is usually not required since the virus need not remain infectious.

- Electron microscopy. For negative contrast EM, the specimen is transported to the laboratory without any additives (dilution!).
- Antigen assay. For an immunofluorescence antigen assay, slide preparations must be made and fixed immediately after sampling. Special extraction mediums are used in EIA. Since commercial kits are used in most cases, procedure and reagents should be correlated with the laboratory.
- Genome hybridization. Here as well, the specimen material must meet specific conditions depending on whether the viruses are to be identified by the in-situ method or after extraction. This must be arranged beforehand with the laboratory.

Significance of results. A positive result with a direct virus detection method has the same level of significance as virus isolation. A negative test result means very little, particularly with EM, due to the low level of sensitivity of this method. The antigen assay and genome hybridization procedures are more sensitive than EM, but they are selective and detect only the viruses against which the antibodies or the nucleic acid probe used, are directed. It is therefore of decisive importance to provide the laboratory with detailed information. (See p. 208f. for definitions of the terms *sensitivity* and *specificity*.)

Virus Detection Following Biochemical Amplification

Polymerase chain reaction (**PCR**, Fig. 7.**9**). This method provides a highly sensitive test for viral genomes. First, nucleic acid is extracted from the patient material to be analyzed. Any RNA virus genome present in the material is transcribed into DNA by reverse transcriptase (see p. 385f.). This DNA, as well as the DNA of the DNA viruses, is then replicated in vitro with a DNA polymerase as follows: after the DNA double strand has been separated by applying heat, two synthetic oligonucleotides are added that are complementary to the two ends of the viral genome segment being looked for and can hybridize to it accordingly. The adjacent DNA (toward each 5′ end) is then

Polymerase Chain Reaction

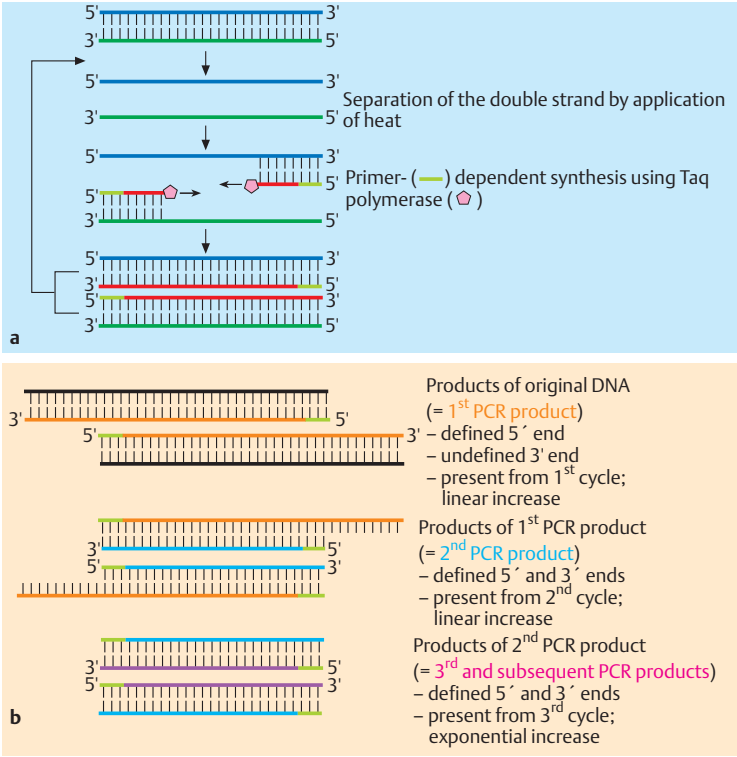


Fig. 7.**9** **a** Two oligonucleotide primers are hybridized to the DNA double strands, which have been separated by heating. A heat-stable polymerase (e.g., *Taq* polymerase from ***T**hermus **aq**uaticus*) is then added and extends these primers along the length of, and complementary to, the matrix strand. The resulting double strands are then once again separated by heat and the reaction is repeated.
b The DNA strands produced in the first cycle (1st generation) have a defined 5′ end (corresponding to the primer) and an undefined 3′ end. All of the subsequent daughter strands (2nd to nth generation) have a uniform, defined length.

copied with an added polymerase, whereby the oligonucleotides act as primers. The new and old strands are once again separated by heat and the reaction is started over again. Running several such cycles amplifies the original viral DNA by a factor of many thousands. Beginning with the second generation, the newly synthesized DNA strands show a uniform, defined length and are therefore detectable by means of gel electrophoresis. The specificity of the reaction is verified by checking the sequences of these DNA strands by means of hybridization or sequencing. The amplification and detection systems in use today for many viruses are increasingly commercially available, and in some cases are also designed to provide quantitative data on the "viral load."

Serodiagnosis

If a viral infection induces humoral immunity (see p. 48f. and 401), the resulting antibodies can be used in a serodiagnosis. When interpreting the serological data, one is confronted by the problem of deciding whether the observed reactions indicate a fresh, current infection or earlier contact with the virus in question. Two criteria can help with this decision:

Detection of IgM (without IgG) proves the presence of a fresh primary infection. IgM is now usually detected by specific serum against human IgM in the so-called capture test, an EIA (p. 128).

To test for IgM alone, a blood specimen must be obtained very early in the infection cycle. Concurrent detection of IgG and IgM in blood sampled somewhat later in the course of the disease would also indicate a fresh infection. It could, however, also indicate a reactivated latent infection or an anamnestic reaction (i.e., a nonspecific increase in antibodies in reaction to a nonrelated infection), since IgM can also be produced in both of these cases.

A **fourfold increase in the IgG titer** within 10–14 days early on in the course of the infection or a drop of the same dimensions later in the course would also be confirmation.