Viral Proliferation and Replication

3

Contents

3.1   How Is the Infection of a Cell Initiated?

As obligate cellular parasites, viruses do not have their own metabolism; therefore, they must infect cells for reproduction. The virus particles must be able to recognize specific receptor molecules on the cytoplasmic membrane of the host cell and to bind to them. This process is known as attachment. In enveloped viruses, this interaction is mediated by proteins that are embedded within the viral envelope. This is the case in influenza viruses as well as retroviruses and herpesviruses. Binding of viral envelope proteins to cellular surface structures is to some extent very specific: this is the case for the interaction between the surface protein gp120 of human immunodeficiency virus (HIV) and the CD4 receptor, a polypeptide that occurs almost exclusively in the cytoplasmic membrane of T-helper cells and macrophages (▶ Sect. 18.1.5). In other cases, viral proteins bind to cellular structures that are found on various cell types. One example is the binding of haemagglutinin of influenza viruses (▶ Sect. 16.3) to terminal *N*-acetylneuraminic

acid residues of complex oligosaccharides, which are found as protein and lipid modifications on the membrane surface of various cells.

In non-enveloped viruses, the surface of the capsid proteins contains the structures that are responsible for the more or less specific binding of the particles to particular cells. Picornaviruses, adenoviruses and parvoviruses are such examples. Polioviruses interact with a domain of CD155, a protein of the immunoglobulin superfamily that is localized on the cell surface, by the canyon – a trench-like structure formed by the folding of specific amino acid regions of the capsid protein on the surface of the particle. Most rhinoviruses, which also belong to the picornavirus family, use the cell surface protein intercellular adhesion molecule 1 for specific attachment; other rhinoviruses binds to members of the LDL receptor family (▶ Sect. 14.1.4). Adenoviruses attach to the coxsackievirus and adenovirus receptor, a functionally not characterized cell surface protein, by the knob at the end of the fibre proteins, which are located at the vertices of the icosahedral capsids. The receptor is denominated so because both virus types bond to it (▶ Sects. 14.1.4 and ▶ 19.4.4). The simultaneous interaction of adenoviral penton base proteins, also components of the particle surface, with $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrins is also necessary for successful attachment (▶ Sect. 19.4.4). This imperative to bind to two different receptor types for successful infection of target cells is also found in other viruses, e.g. in human immunodeficiency viruses and herpesviruses (▶ Sects. 18.1 and ▶ 19.5).

3.2   How Does a Virus Enter a Cell?

After attachment, virus particles that are associated with their respective receptor on the cell surface are translocated into the interior of the cell; this process is referred to as penetration. In non-enveloped viruses, this is usually performed by receptor-mediated endocytosis. This process is generally used by cells to incorporate molecules from the outside into the cytoplasm: the capsid-receptor complexes interact with clathrin-rich membrane sites, where the cytoplasmic membrane invaginates around the bound virus, enclosing it. The resulting vesicle is referred to as an endosome. It invaginates inwards, thus entering the cytoplasm of the cell. Alternative entry routes are through caveolae and caveosomes. For the next steps of the infection cycle, the virus particles must be released relatively quickly from endosomes because these are rich in proteases and other degrading enzymes, which would eventually destroy the virus. Therefore, viruses have evolved mechanisms that allow them to leave the vesicle and to avoid the further endocytosis and degradation process. For such purposes, parvoviruses (▶ Sect. 20.1) possess phospholipase A_2 like enzyme activities, which as part of a capsid protein (VP1) are responsible for release of the virus from the endosome.

Even enveloped viruses have developed ways to escape destruction in the endosomes, as they also penetrate into the cell in part by endocytosis of membrane vesicles. For example, if influenza viruses penetrate a cell (▶ Sect. 16.3), the capsid is surrounded by two membranes in the vesicles, namely the viral envelope and the

vesicle membrane that is derived from the plasma membrane. A fusionally active sequence of the viral haemagglutinin triggers the fusion of the two membranes, leading to the release of the virus particle from the vesicles. This process is found in similar variants in many other enveloped viruses, e.g. in flaviviruses (▶ Sect. 14.5). The fusion of the two membranes is pH-dependent; therefore, acidification of the vesicle interior must occur previously. In contrast to this penetration mechanism, paramyxoviruses (▶ Sect. 15.3) possess in their envelope a special fusion protein that makes possible, in a pH-independent process, the fusion of the viral envelope and cellular membranes during attachment of the particle; in this case, the capsid is released directly into the cytoplasm after merging of the two membranes. The fusion of the viral envelope with the cell membrane is also performed in a similar manner in herpesviruses (▶ Sect. 19.5) and HIV (▶ Sect. 18.1).

3.3   How Is the Viral Genome Released into the Cell?

The release of the viral nucleic acid from the capsid is the result of a still largely unsolved process, which is referred to as uncoating. During this process, the genome of DNA viruses – in herpesviruses also the tegument – is transported by different intracellular transport systems through the nuclear pores into the nucleus. An exception is poxviruses (▶ Sect. 19.6), which replicate as DNA viruses in the cytoplasm of infected cells. After uncoating, the genome of RNA viruses remains as a ribonucleic acid–protein complex in the cytoplasm, where the next steps of the infection cycle occur. This rule is broken only by influenza viruses and Borna disease virus, which replicate as RNA viruses in the nucleus (▶ Sects. 15.2 and ▶ 16.3).

3.4   What Different Strategies Do Viruses Pursue in Gene Expression and Genome Replication?

Viral replication outlines the very complex processes of viral gene expression and genome replication, which are different in all virus types, and finally result in the production of multiple copies of the virus in infected cells. Although viruses possess the genetic information for most of the factors required for their own gene expression and genome replication, specific cellular proteins are often essential for viral gene expression. These proteins usually act as transactivators of viral gene transcription. If they are lacking, the infection cycle cannot continue; the result is that no or only a subset of the viral gene products are synthesized, and the formation of infectious particles remains incomplete. This form of infection, in which the virus is capable of binding to the surface of certain cells and penetrating into them, cannot initiate (or can initiate only partially) the reproductive cycle because of the given intracellular conditions, is referred to as abortive infection. The dependence of replication on the cellular environment is another reason for the cell specificity of viral infections.

Depending on the nature and structure of the viral genome, the replication strategies described in the following sections have been found in the different viruses (the chapters in Part II follow this subdivision).

3.4.1   Positive-Sense RNA Viruses

The RNA genomes of positive-sense RNA viruses (▶ Chap. 14) have the polarity of a messenger RNA (mRNA), and can be directly translated into proteins by using the cellular translation machinery. This results in the synthesis of a large precursor polyprotein in picornaviruses and flaviviruses, whereas two or more different forms of the precursor proteins are found in caliciviruses, togaviruses, arteriviruses and coronaviruses. They are proteolytically cleaved into the viral structural proteins and enzymes. One of the enzymes is very important for genome replication, namely RNA-dependent RNA polymerase. Since this enzyme does not exist in eukaryotic cells, the virus must encode the corresponding genetic information itself. Using the positive-sense RNA genome as a template, this polymerase catalyses the synthesis of a complementary negative-sense RNA, which in turn serves as a template for the production of a large number of new RNA genomes with positive-sense polarity. Important prototypes of positive-sense RNA viruses are picornaviruses, flaviviruses, togaviruses and coronaviruses (▶ Sects. 14.1, ▶ 14.5, ▶ 14.6 and ▶ 14.8).

3.4.2   Negative-Sense RNA Viruses

In contrast to positive-sense RNA viruses, the genome of negative-sense RNA viruses (▶ Chaps. 15 and ▶ 16) does not exhibit the polarity of mRNA; hence, it cannot be directly translated into proteins. This requires transcription of the genome into a complementary RNA molecule, a process that relies on the presence of an RNA-dependent RNA polymerase. Since viral proteins cannot be synthesized directly from the genome owing to its negative-sense polarity, negative-sense RNA viruses must carry this polymerase into the cell as part of the virus particle. The enzyme synthesizes complementary mRNA molecules, which are then translated into viral structural and non-structural proteins. In the following step, the RNA-dependent RNA polymerase is also responsible for the synthesis of a continuous, complementary RNA strand, which serves as a template for the synthesis of negative-sense RNA genomes. Important prototypes of negative-sense RNA viruses are rhabdoviruses and paramyxoviruses (▶ Sects. 15.1 and ▶ 15.3) as well as orthomyxoviruses, which differ from the afore-mentioned viruses by a segmented negative-sense RNA genome (▶ Sect. 16.3). Some virus types that belong to the bunyavirus and arenavirus families (▶ Sects. 16.1 and ▶ 16.2) have segmented genomes as well, and can use parts of them in both positive-sense and negative-sense polarity. Their single-stranded RNA genomes encode proteins in both directions. This highly

efficient use of the coding capacity is referred to as ambisense orientation. It is also found in porcine circovirus, which has a single-stranded, circular DNA genome (▶ Sect. 20.2).

3.4.3   Double-Stranded RNA Viruses

Reoviruses and birnaviruses (▶ Chap. 17) have a double-stranded, segmented RNA genome. In this case, an RNA-dependent RNA polymerase is also found as part of the virus particles, and is carried into the cell during infection. It transcribes the negative-sense genomic fragments into capped, translatable mRNA molecules. These also serve as templates for the synthesis of new double strands. Only reoviruses and birnaviruses follow this principle of conservative replication, in which none of the parent strands are present in the newly synthesized double-stranded RNA molecules.

3.4.4   Retroviruses

These single-stranded RNA viruses (▶ Chap. 18) have a positive-sense genome, but their replication cycle differs completely from that of the previously mentioned virus families. Retroviruses enclose the enzyme reverse transcriptase in their virions, and this is introduced into the cell during infection. The RNA-dependent DNA polymerase activity of reverse transcriptase catalyses the transcription of the RNA genome template into double-stranded DNA, which is subsequently integrated into the cellular genome. This so-called provirus behaves like a normal cellular chromosomal region; it is replicated along with the cellular genome during cell division and is passed on to daughter cells. Transcription and translation occur only from the integrated viral DNA. This produces spliced and unspliced mRNA molecules that are translated into viral structural proteins and enzymes. The unspliced sequence of the provirus spanning the entire mRNA serves as a viral genome, which is packaged into virus particles.

3.4.5   Double-Stranded DNA Viruses

The genome of these viruses (▶ Chap. 19) is transcribed by cellular enzymes after transport into the nucleus. The resulting RNA molecules are then translated into the viral non-structural and structural proteins. Hepadnaviruses (▶ Sect. 19.1) have a partially double-stranded DNA genome, which is completed in the infected cells and is present in the nucleus as a circular molecule. Hepadnaviruses have a reverse transcriptase, which emphasizes, along with some other attributes, their relationship to retroviruses. During the replication cycle, the mRNA that spans the entire genome is transcribed into DNA by this viral enzyme. The smaller DNA viruses such as polyomaviruses (▶ Sect. 19.2) do not encode their own DNA polymerase,

but encode polypeptides that interact with the cellular DNA polymerases, and alter the function of these in such a way that the viral DNA sequences are preferentially replicated. This process begins at the origin of replication and proceeds bidirectionally and semiconservatively; it is very similar to delta or plasmid replication, which is found during replication of circular bacterial chromosomes or episomal DNA molecules.

The more complex DNA viruses such as adenoviruses and herpesviruses (▶ Sects. 19.4 and ▶ 19.5) have a tightly regulated gene expression pattern that is divided into early and late phases; these viruses use the cellular transcription and translation machinery as well. Several regulatory and enzymatically active polypeptides are synthesized early, including the viral DNA polymerases and some enzymes that are involved in nucleic acid metabolism, which make possible the replication of double-stranded DNA genomes. The linear genomes of adenoviruses are replicated in a semiconservative mode; this means that each parent strand is used as a template and remains as part of the newly synthesized double-stranded DNA molecules. The replication origins are located at the ends of the double-stranded DNA. The linear DNA genomes of herpesviruses are circularized in the cell. These viruses can have two different replication cycles: during latency, the viral DNA is present as an episome and is replicated by cellular DNA polymerases. In the lytic infection cycle, which leads to the production of progeny viruses, replication occurs according to the principle of sigma replication, which also occurs in some bacteriophages, and is referred to as rolling-circle replication. In this replication mode, a strand of the circular DNA molecule is cleaved at the origin of replication, generating a free $3'$-OH end, which is continuously extended by polymerization of further nucleotides by the viral DNA polymerase, whereby the intact DNA strand serves as a template. The $5'$ end is continuously displaced from the template strand, just as if it were rolled. In this way, a single strand of DNA is generated that encompasses multiple copies of the herpesvirus genome in concatemeric form, i.e. repeated in series. It is converted to a double DNA strand by discontinuous synthesis of Okazaki fragments, and is cleaved by endonucleases that resolve the concatemers into individual viral genomes. In both adenoviruses and herpesviruses, the synthesis of viral structural proteins is induced only in the late phase of gene expression after DNA replication.

Poxviruses are also double-stranded DNA viruses, but they follow a completely different replication mode. They perform all synthesis reactions in the cytoplasm of infected cells. Therefore, all enzymes that are usually localized in the nucleus cannot be used by poxviruses. These include RNA polymerases, capping enzymes and RNA-modifying enzymes. Therefore, poxviruses possess, in addition to their own DNA polymerase, also the genetic information for these functions. Gene expression and genome replication are also tightly regulated in poxviruses (▶ Sect. 19.6). The asfarvirus family includes only one animal pathogenic agent: African swine fever virus (▶ Sect. 19.7). Asfarviruses have a double-stranded DNA genome and are similar to the phytopathogenic iridoviruses in many ways. The replication occurs in the nucleus of infected cells.

3.4.6   Single-Stranded DNA Viruses

The parvovirus, anellovirus and circovirus families encompass viruses with a single-stranded linear or circular DNA genome (▶ Chap. 20). All three families do not encode a viral DNA polymerase; like polyomaviruses, they also use cellular enzymes for genome replication, and these are functionally modified. In this way, complementary double-stranded DNA intermediates are generated that are subsequently transcribed into single-stranded genomes (▶ Sects. 20.1 and ▶ 20.2).

3.5   What Is Morphogenesis?

After replication, both the viral structural proteins and the viral genome are present in multiple copies in the cell. The process of viral morphogenesis describes the orderly assembly of the various components into particulate structures, capsids and ultimately infectious virus particles. Assembly occurs largely without the use of cellular enzymes and other enzymatic activities by interaction between the individual components, and hence is also known as self-assembly. However, there are growing indications that viral morphogenesis cannot proceed entirely without the involvement of cellular functions. Viral proteins, above all cellular chaperones (protein folding catalysts), influence the self-assembly process. The morphogenesis of enveloped viruses is frequently associated with cellular membrane structures. In retroviruses, for example, morphogenesis occurs at the cytoplasmic membrane; however, in herpesviruses, it occurs initially at the inner nuclear membrane, and later on the membranes of the *trans*-Golgi network; in flaviviruses, it occurs at the membrane of the endoplasmic reticulum (▶ Sects. 18.1, ▶ 19.5 and ▶ 14.5).

3.6   How Are Progeny Viruses Released?

One possible way of releasing infectious particles is budding. Here, the preformed capsids aggregate at specific sites in the cytoplasmic membrane, the lipid rafts. In these membrane islands, viral surface proteins accumulate after their transport. The assembled capsids are subsequently enveloped by the protein-containing membrane and are finally released by budding. Depending on the site of the assembly process in the cell, the viral envelope originates from the cytoplasmic membrane, the nuclear membrane, the endoplasmic reticulum membrane or the membrane of the *trans*-Golgi network. If the viral envelope originates from the cytoplasmic membrane, viruses will be directly released into the surrounding region. If viral morphogenesis occurs at the nuclear membrane or at the membrane of the endoplasmic reticulum, release of the virus occurs via transport through the Golgi apparatus to the cell surface and by exocytosis. Some viruses, e.g. HIV, can still perform maturation processes by structural rearrangements in the particles after having been released from the cells. The release of non-enveloped viruses predominantly occurs by lysis of the infected cell. Whether this is an active process induced by the

virus or whether viral replication and the associated interference with the cellular metabolism lead to exhaustion of the cell to the extent that it induces apoptosis resulting in cell death and disintegration is largely unknown and possibly proceeds very differently in the various virus systems.

Replication of viruses, their morphogenesis and their release imply many errors that may lead to the formation of non-infectious, defective virus particles. Defective viruses frequently arise in excess. In many cases, they contain an incomplete replicated genome; in other cases, the loss of infectivity is based on irregular processes during assembly and subsequent viral maturation.

Further Reading

Cann AJ (2005) Principles of molecular virology, 4th edn. Academic, Burlington
Doerfler W, Böhm P (1993) Virus strategies. Molecular biology and pathogenesis. VCH, Weinheim
Flint SJ, Enquist LW, Krug RM, Racaniello VR, Skalka AM (2004) Principles of virology. Molecular biology, pathogenesis, and control, 2nd edn. ASM Press, Washington
Knippers R (2006) Molekulare Genetik, 9th edn. Thieme, Stuttgart
Lewin B (2007) Genes IX. Jones & Bartlett, Sudbury