

Chapter 15

Virus-Receptor Interactions and Receptor-Mediated Virus Entry into Host Cells

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Abstract The virus particles described in previous chapters are vehicles that transmit the viral genome and the infection from cell to cell. To initiate the infective cycle, the viral genome must therefore translocate from the viral particle to the cytoplasm. *Via* distinct proteins or motifs in their outermost shell, the particles attach initially to specific molecules on the host cell surface. These virus receptors thus mediate penetration of the viral genome inside the cell, where the intracellular infective cycle starts. The presence of these receptors on the cell surface is a principal determinant of virus host tropism. Viruses can use diverse types of molecules to attach to and enter into cells. In addition, virus-receptor recognition can evolve over the course of an infection, and virus variants with distinct receptor-binding specificities and tropism can appear. The identification of virus receptors and the characterization of virus-receptor interactions have been major research goals in virology for the last two decades. In this chapter, we will describe, from a structural perspective, several virus-receptor interactions and the active role of receptor molecules in virus entry.

Keywords Virus-host • Virus tropism • Virus attachment • Virus-receptor • Virus structure • Virus entry • Virus neutralization • Capsid dynamics • Uncoating • Membrane penetration • Endocytosis • Cell surface • Cell adhesion • Cell surface molecules • Membrane proteins • Glycoproteins • Carbohydrates • Protein interactions • Molecular recognition • Crystallography • Cryo-EM

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Abbreviations

Ad	Adenovirus
APN	Aminopeptidase N
CAR	Coxsackievirus-adenovirus receptor
cryo-EM	Cryo-electron microscopy
CV	Coxsackievirus
D	Domain
DAF	Decay-accelerating factor
DC	Dendritic cells
DC-SIGN	(DC-specific ICAM-3-grabbing nonintegrin)
EFN	Ephrin
EV	Echovirus
FMDV	Foot-and-mouth disease virus
g/gp	Glycoprotein
H	Hemagglutinin
HA	Influenza A hemagglutinin
HeV	Hendra virus
HIV-1	Human immunodeficiency virus-type 1
HN	Hemagglutinin neuraminidase
HRV	Human rhinovirus
HS	Heparan sulphate
HSV	Herpes simplex virus
ICAM-1	Intercellular adhesion molecule-1
IgSF	Immunoglobulin superfamily
LDLR	Low-density lipoprotein receptor
MCP	Membrane cofactor protein
MV	Measles virus
N	Neuraminidase
NAG	N-acetyl-glucosamine
NDV	Newcastle disease virus
NiV	Nipah virus
PM	Plasma membrane
PV	Poliovirus
PVR	Poliovirus receptor
SCR	Short consensus repeats
SLAMF	Signalling lymphocytic activation molecule
VP	Viral capsid protein.

15.1 Introduction: Virus Entry into Host Cells, the Recognition of Cell Surface Molecules

The viral particles formed in infected host cells (see Chaps. 10, 11, 12, 13, and 14) are metastable structures that transmit the viral genome and the infection from cell to cell. Viruses must therefore penetrate host cells to initiate the replicative infective cycle by exploiting the cell machinery. In the extracellular transit stage of the viral cycle, animal viruses and bacteriophages attach to specific cell surface molecules (virus receptors) suited for host cell entry following virus-specific entry pathways. Virus receptors must be distinguished from attachment factors, surface molecules to which some viruses can bind but that do not themselves promote virus entry into host cells [1]. Virus binding to attachment factors concentrates virus particles onto the cell surface, which can help viruses to encounter specific entry receptors that mediate genome translocation into the cytoplasm. The virus receptor molecules are not just required for initial virus binding to host cells, but also for the transfer of the viral genome through cellular membranes [1]. Virus-receptor interactions can trigger changes in the virus particles that initiate genome translocation, or alterations in the cell, such as signalling events that facilitate virus entry [2]. Viruses enter host cells at the cell *surface* or after endocytosis (Fig. 15.1). Multivalent binding of the virus particles to receptors on the cell surface can mediate uncoating or release of the viral genome in non-enveloped viruses and/or fusion of the virus and cell membranes (Fig. 15.1). Moreover, viruses bound to cell surface molecules can be internalized by following different endocytic pathways [3], where exposure to low pH, enzymatic modification or other cellular factors leads to the delivery of the genome into the cytoplasm (Fig. 15.1).

Viruses evolve to recognize specific cell surface receptor molecules appropriate for productive entry and infection of host cells, which frequently determines the host tropism or the cell type a virus can infect. Selection of cell entry receptor by viruses appears to be determined by subtle interactions that regulate the specificity and affinity necessary for efficient cell attachment. Virus-receptor interaction can nonetheless be a highly dynamic process. A single virus can recognize one or several cell entry molecules, which can also differ among virus variants or during the course of an infection [4, 5]. Virus recognition of receptors is under continuous evolutionary pressure to increase their infection efficiency, which can lead to the emergence of virus variants with altered infectivity or tissue tropism.

In this chapter, several examples of animal virus-receptor interactions will be presented, together with a description of known models of receptor-switching viruses. The chapter presents a structural view of some virus-receptor interactions that have been characterized by structures of complexes. We will also describe how the viral genome exits the capsid (uncoating) in some non-enveloped animal viruses, illustrating the role of cell surface receptor molecules in the entry process. Membrane penetration events in enveloped viruses are discussed in Chap. 16. Specific aspects of receptor recognition and injection-mediated genome uncoating by some bacteriophages are described in Chap. 17.

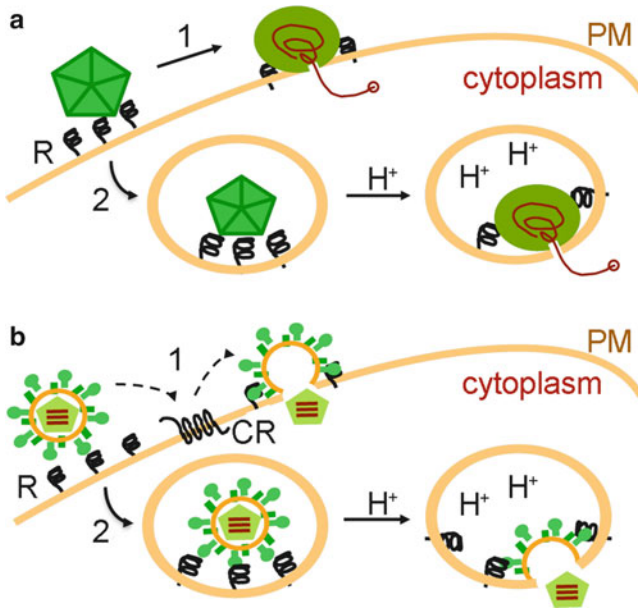


Fig. 15.1 Virus entry into host cells. Scheme illustrating two cell entry pathways by non-enveloped (a) or enveloped viruses (b). pH-independent entry at the cell surface (*pathway 1*): Genome uncoating and penetration into cytoplasm (a) or virus-cell membrane fusion (b) at the plasma membrane (PM). Virus binding to a single receptor (R) or to additional co-receptor molecules (CR) at neutral pH triggers genome translocation. pH-dependent entry in endosomes (*pathway 2*): Receptor-mediated endocytosis of viruses prior to penetration into the cytoplasm. In the endosomes, viruses firmly bound to multiple receptor molecules are exposed to progressively lower pH, which triggers genome uncoating and penetration (a) or fusion (b). The entry reaction is mediated by acidification of endosomal compartments, with or without receptor priming

15.2 Virus-Receptor Interactions and Receptor Specificity Switch

The known number and diversity of cell surface molecules exploited by viruses to enter host cells is still increasing [6]. Viruses recognize a variety of cell surface molecules specifically, including glycolipids, carbohydrates and proteins with very distinct folding structures. There are viruses specific for a single receptor molecule, whereas others bind to several structurally distinct receptors for host cell entry [2]. The virus particles use certain proteins in their outermost shell to attach to the cell surface molecules. The multivalent nature of the particles allows viruses to bind with very high avidity to the host cell surface, even though monomeric virus-receptor interactions are usually of moderate affinity (0.1–1 μM) [7].

Some non-enveloped viruses have proteins specialized in attachment to cell surface receptors; however, in other cases unique motifs on the naked capsids are engaged in receptor recognition. Here we discuss both receptor recognition modes,

providing a description of adenovirus and picornavirus binding to cell surface molecules. The enveloped viruses bear membrane-bound glycoproteins that bind to the receptor and trigger virus-cell membrane fusion during the entry process, as will be described in Chap. 16. We present the diverse types of cell surface receptors recognized by human immunodeficiency virus and paramyxoviruses, which have been characterized. In the last part of this section, we illustrate the importance of cell surface carbohydrates in virus attachment and infection of host cells.

15.2.1 Virus-Receptor Interactions in Non-Enveloped Viruses

In this subsection, we describe receptor recognition by two families of non-enveloped viruses (picornaviruses and adenoviruses) for which virus-receptor interactions have been characterised in detail by structural studies. Picornaviruses use their naked capsid to attach to receptors, whereas adenoviruses have fibres that protrude from the capsid for attachment to several cell surface molecules.

Picornaviruses and Their Receptors

The picornaviruses are a large family of non-enveloped viruses responsible for numerous human and animal diseases. Picornavirus particles are formed by an icosahedral protein capsid built by three external viral capsid proteins (VP1 to VP3) and the internal protein VP4 packed inside with a single-stranded RNA genome (Fig. 15.2a). The capsid is composed of 60 basic subunits or protomers arranged as 12 pentamers (see Chap. 10). The members of this virus family bind to distinct types of receptor molecules suited for entry into host cells [8].

The poliovirus receptor (PVR) was one of the first picornavirus receptors to be characterized [9]. PVR is a type I membrane protein and a member of the immunoglobulin superfamily (IgSF), which has three Ig-like domains (D1 to D3) at the extracellular region (Fig. 15.2a). The receptor for most (90 %) identified human rhinovirus (HRV) serotypes, the major group of HRV, is intercellular adhesion molecule-1 (ICAM-1) [10, 11], another IgSF member with five Ig-like extracellular domains (Fig. 15.2a). PV and HRV bind similarly to the N-terminal membrane distal domains of the receptor molecules (Fig. 15.2b) [8]. Both viruses use a depressed surface, or canyon, formed by two neighbouring protomers around the five-fold icosahedral vertices of the capsid (Figs. 15.2a, b). Cryo-electron microscopy (cryo-EM; see Chap. 3) and binding studies show some differences in the way PV and HRV bind to their IgSF receptors. Kinetics for monomeric receptor binding to the virus particles showed more rapid kinetic binding rates to PV than to HRV [12]. Moreover, cryo-EM structures of virus-receptor complexes showed that PV uses more exposed residues on the canyon walls than HRV [8, 12].

The use of the canyon for binding to cell surface receptors was also described for other picornaviruses such as the coxsackieviruses (CV) A21 and B3, and echovirus 1 (EV1) [8, 13]. CVA21 binds to ICAM-1, CVB3 to the coxsackievirus-adenovirus receptor (CAR), and echovirus 1 (EV1) to the $\alpha_2\beta_1$ integrin. The CAR protein also

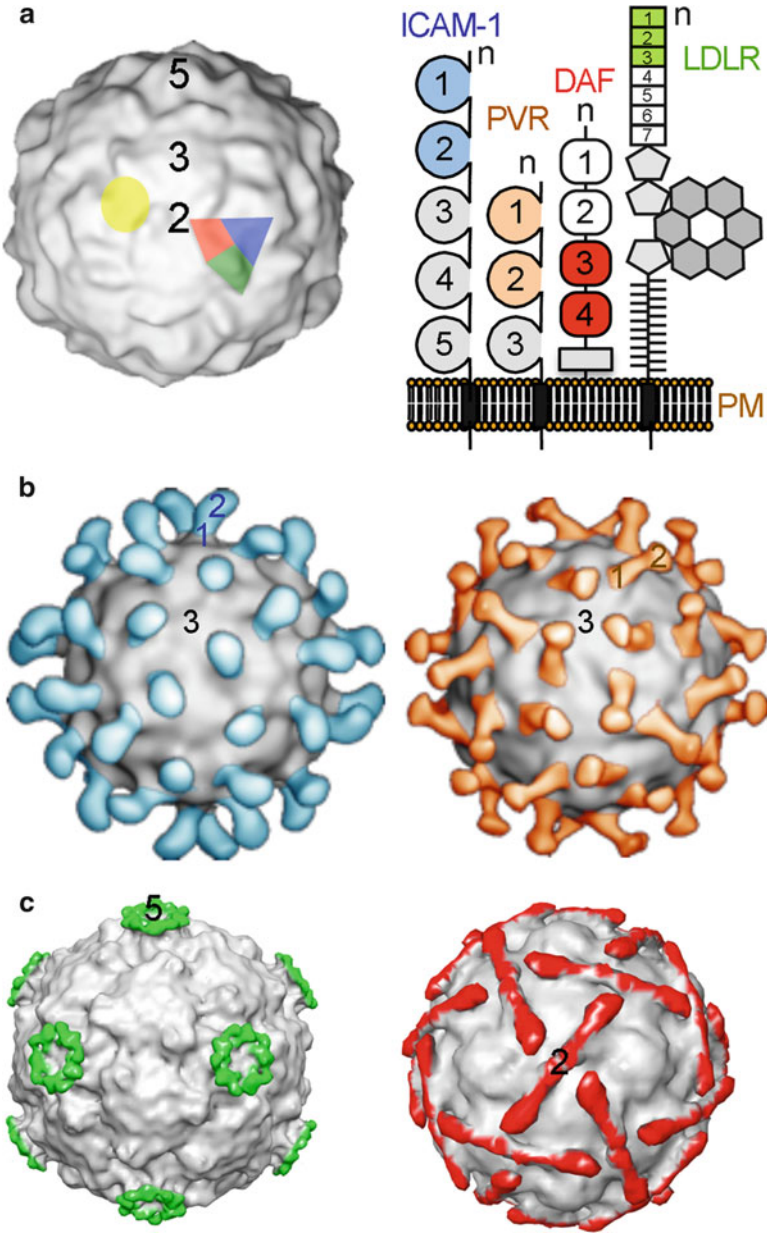


Fig. 15.2 Virus-receptor interactions in picornaviruses. (a) *Left*: Surface representation of the poliovirus (PV) particle computed from the crystal structure of PV1/M (PDB ID 1ASJ). Numbers indicate location of the icosahedral two-, three- and five-fold axes. The *triangle* illustrates the approximate location of a capsid building block or protomer, assembled from the external VP1 (*blue*), VP2 (*green*) and VP3 (*red*). The canyon region is marked with a *yellow circle*. *Right*: Scheme of picornavirus receptors ICAM-1, PVR, DAF and LDLR. The N-terminus (n) and

belongs to the IgSF, but the $\alpha_2\beta_1$ integrin is a non-IgSF member that fits into the picornavirus canyon. Cryo-EM structural studies showed that the I-domain of the integrin penetrates the EV1 capsid canyon [13], as described for PV, HRV and CVA21 binding to IgSF receptor molecules. Thus, the canyon is a well-defined receptor-binding region in picornaviruses [8]. The concave nature of the canyon is thought to be suited for hiding conserved receptor-binding residues from neutralizing antibodies, which cannot penetrate the depressed surface as efficiently as relatively narrower receptor molecules. This hypothesis was nonetheless challenged by studies showing an antibody that penetrates the canyon [14], although it interacts mostly with the walls rather than the bottom of the canyon. Receptor-binding residues in PV are more exposed on the canyon walls and can thus be targeted by antibodies elicited by PV vaccines [8, 12]. The use of recessed virus surfaces in receptor recognition is relatively common in virus-receptor interactions, and could be a viral strategy to protect some receptor-binding residues from immune surveillance and/or might have arisen to increase surface contact area and binding energy for cell receptor molecules.

Other picornaviruses do not use the canyon for attachment to cell entry receptors; viruses of this family, such as foot-and-mouth disease virus (FMDV), some HRV and echoviruses (EV), use exposed regions of the capsid. FMDV exploits an RGD motif exposed in the VP1 GH loop to bind to integrin receptors [15]. This receptor-binding motif is accessible and is a major antigenic site; however, virus can escape from immune neutralization by mutating some residues adjacent to the RGD, which prevents detection by some antibodies while preserving cell attachment activity and viability. Approximately 10 % of HRV serotypes, the minor group, do not bind to ICAM-1, and use members of the low density lipoprotein receptor (LDLR) family to enter host cells (Fig. 15.2a) [16]. Even though minor and major group HRV are closely related, the mode by which they bind their respective receptors is strikingly different (Figs. 15.2b, c). Minor group HRV do not use the canyon, and contact the receptor through a protruding region close to the capsid five-fold axis (Fig. 15.2c, left) [17]. These HRV bear a conserved Lys residue in VP1 that contacts an acidic cluster and a Trp residue in the N-terminal ligand binding domains of the LDLR proteins. This lysine is absent in major group HRV that bind to ICAM-1, and is therefore likely to be a major determinant of the distinct receptor-binding specificity described for rhinoviruses.



Fig. 15.2 (continued) extracellular domains are labelled. Receptor domains used to determine structures of virus-receptor complexes shown below are coloured. **(b)** Cryo-EM structure of HRV3-ICAM-1 (*left*) and PV1-PVR (*right*), picornaviruses that use the canyon for receptor binding. The complexes were prepared in solution with purified virus particles and two-domain (1 and 2) fragments of the receptor molecules. Location of the domains is shown. D1 penetrates the canyon, whereas D2 does not contact the virus and protrudes from the capsid. Images provided by Holland Cheng and Li Xing, adapted from references [12, 79]. **(c)** Cryo-EM structure of HRV2-LDLR (EMD-1049) and EV12-DAF (EMD-1057) complexes, representative of picornaviruses that do not use the canyon for receptor binding. Ligand binding repeats 1–3 of the very low-density lipoprotein receptor or domains 3–4 of DAF were used to prepare complexes. Surfaces of bound receptors are coloured as in **(a)**. Images prepared with Chimera (cgl.ucsf.edu/chimera) from cryo-EM maps

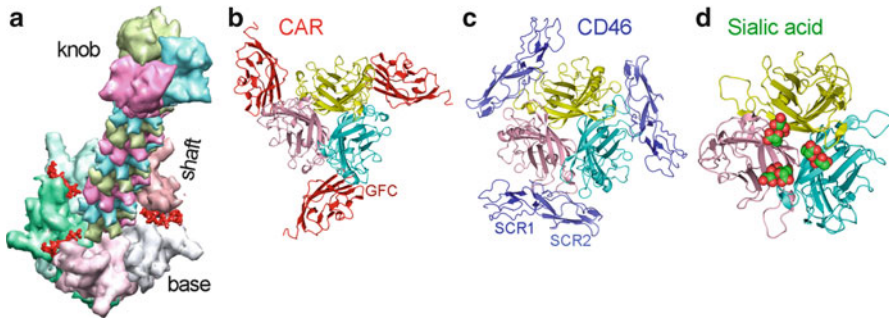


Fig. 15.3 Virus-receptor interactions in adenoviruses. Representation of the receptor-binding adenovirus fibre and penton base (a) (see also Chap. 11), and crystal structures of the trimeric Ad knob in complex with CAR (b), CD46 (c), or sialic acid (d). Ribbon drawings are shown for the complexes prepared with the N-terminal (D1) of CAR (red) and Ad12 (PDB ID 1KAC), or with the two N-terminal short consensus repeats (SCR1, 2) of CD46 (blue) and Ad11 (PDB ID 2O39). The Ad-binding GFC β -sheet of the CAR domain is indicated. Sialic acids in complex with the Ad37 knob (PDB ID 1UXA) are shown with carbons (green) and oxygens (red) as spheres. Figure (a) was provided by Carmen San Martín and figures (b–d) were prepared with PyMOL (pymol.org)

A picornavirus-receptor interaction different from those described above was reported for EV using decay-accelerating factor (DAF, CD55) as a cell entry receptor [18]. These viruses bind to DAF through a capsid protrusion at the southern rim of the canyon, distant from the five-fold axis and near the capsid two-fold axis (Fig. 15.2c, right) [19, 20]. The receptor-binding region in EV11 protrudes and is more exposed than the canyon. The kinetic association rate for the monomeric EV11-CD55 interaction is thus one to two orders of magnitude higher than those shown for picornaviruses that use the canyon for receptor recognition, which probably reflects the distinct nature of the virus-receptor interactions [21].

The structural studies of picornavirus-receptor complexes discussed here show that viruses of this family can use diverse capsid regions for recognition of cell surface molecules and for productive entry into host cells.

Adenoviruses, Non-enveloped Viruses That Bind to Several Cell Surface Molecules

Adenoviruses (Ad) are non-enveloped viruses with icosahedral capsids built by hexon and penton capsomers (see Chap. 2 for definition of capsomers and Chap. 11 for additional information and references). The pentons locate at each of the 12 icosahedral vertices of the capsid, and forms the base of protruding trimeric fibres (Fig. 15.3a). The penton base and the associated fibres form a complex that recognises cell surface receptor molecules [22, 23]. The most capsid-distal, globular region of the fibre, the knob (Fig. 15.3a), mediates initial Ad attachment to the cell, whereas the penton base is used for subsequent tight attachment of the virus to a secondary receptor that mediates virus internalization and host cell entry [22]. Receptor-bound Ad are transported to endosomes, where acidification triggers capsid disassembly and virion penetration into the cytoplasm.

Cell tropism and receptor recognition is well documented for Ad. This group of non-enveloped viruses can use at least three distinct types of receptor molecules for initial attachment to the cell surface through the fibre proteins [23], which is mediated by the most distal trimeric knob structure (Fig. 15.3). Subgroups A, C, D, E and F attach to the CAR receptor, a member of the IgSF found in epithelial tight junctions. CAR has two Ig-like domains at the extracellular region, and mediates homotypic cell-cell interactions through the same face (GFC β -sheet) of the N-terminal domain engaged by the Ad fibres (Fig. 15.3b) [24, 25]. Fibre binding to CAR interferes with homotypic cell-cell adhesion, which destabilizes epithelial cell layers and facilitates virus release to the airway lumen and spreading to a new host [26].

Most subgroup B Ad use the ubiquitous membrane cofactor protein (MCP, CD46) for initial attachment to the host cell [22]. The Ad knob contacts the two N-terminal short consensus repeats (SCR) of the CD46 molecule (Fig. 15.3c) [27]. Although all Ad use the fibre knob for receptor binding, the mode of contact of the knobs with the CAR or the CD46 proteins is distinct (Fig. 15.3). The switch in receptor-binding specificity between CAR and CD46-binding Ad appear to be related to the distinct conformation of the exposed loops in the periphery of the knob. CD46-binding Ad have an extended knob AB-loop that prevents binding to the CAR receptor, as well as specific structural features in the loops that contact the CD46 molecule [5]. Certain Ad can also attach to cell surface carbohydrates, such as heparin or sialic acid [22, 28, 29]. Subgroup D adenoviruses 8, 19 and 37, which recognize sialic acid, are responsible for epidemic keratoconjunctivitis, a very contagious ocular disease. The sialic acid binding site has been mapped also at the fibre knob, but it locates closer to the centre of the trimer than the sites used to bind to the other receptors (Fig. 15.3c) [28, 29]. The Ad-receptor interaction described here illustrate how different Ad evolved to use distinct knob surfaces to attach to different cell surface receptor molecules; this certainly translates in cell tropism and pathogenesis diversity among the member of this virus family.

Following initial fibre attachment to cell surface receptors, the Ad particles engage cell surface integrin molecules using RGD motifs exposed in loops at the capsid penton base, which is a necessary step for host cell entry by endocytosis [30]. The multivalent interaction of the Ad particle with the integrins mediates its clustering, triggering intracellular signals, rearrangement of actin, and clathrin-mediated endocytosis of the virions [22, 23]. During endocytosis, the virion disassembles and subsequently penetrates the membrane. Ad are thus an example of non-enveloped viruses that use two distinct capsid structures to bind different cell surface receptor molecules for attachment to or penetration of host cells.

15.2.2 Virus-Receptor Interactions in Enveloped Viruses

Enveloped viruses bear membrane-bound glycoproteins specialized in the recognition of cell surface molecules and in subsequent fusion of the viral and cell membranes. The receptor-binding and fusion proteins can be one or two distinct

polypeptides, in many cases resulting from the cleavage of a single precursor protein. Both of these proteins associate in the viral envelope, forming the envelope spikes. Among enveloped viruses, the fusion proteins are less diverse than receptor-binding proteins and will be presented in Chap. 16. Here we will discuss the interactions of some viral envelope spikes with cell surface receptor molecules, described by structures of virus-receptor complexes.

The Human Immunodeficiency Virus Cell Attachment Process

Attachment of the human immunodeficiency virus-type 1 (HIV-1) to cell entry receptors and subsequent fusion have been described in great detail. The HIV-1 particle bears two non-covalently bound viral glycoproteins, gp120 and gp41, which associate to form trimeric envelope spikes [31, 32]. There are around 15 spikes per particle [32]. The gp120 glycoprotein mediates attachment of the HIV-1 particles to cell surface receptors, whereas gp41 catalyses fusion of the virus and the cell membrane, and the release of the nucleoprotein to the cell cytoplasm [31, 33] (see Chap. 16). The HIV-1 entry process is relatively complex [33]. gp120 requires engagement of two distinct cell surface molecules to trigger membrane fusion, which occurs at the cell plasma membrane [34]. gp120 initially engages the lymphocyte-specific CD4 [33], an IgSF cell surface protein. CD4 is a type I membrane protein composed of four Ig-like domains in the extracellular region that forms part of the T cell receptor complex in a subset of T lymphocytes. A depressed groove or pocket in gp120 surrounded by hypervariable loops engages the CD4 N-terminal Ig-like domain with high affinity (Fig. 15.4) [35]. A Tyr residue protruding from the CD4 domain penetrates deeply into the gp120 pocket. The CD4-binding site is surrounded by long hypervariable loops and glycans, which further hide the depressed site from immune surveillance [36].

Binding of gp120 to CD4 triggers conformational changes in the HIV-1 protein that expose a conserved structure (bridging sheet) that binds to a second molecule termed co-receptor [35]. Engagement of the co-receptor molecule is essential for virus-cell membrane fusion and for HIV-1 penetration into the host cell. Two distinct HIV-1 co-receptors have been identified, CXCR4 and CCR5, which are chemokine receptors containing seven transmembrane segments [37]; HIV-1 interaction with CCR5 and CXCR4 is strain-specific. HIV-1 strains using CCR5 (R5 viruses) are mainly associated to sexual transmission. They can also evolve to infect T cells by acquiring the ability to use the CXCR4 receptor (X4 viruses). HIV-1 viruses that use both co-receptors are termed R5X4 viruses.

HIV-1 particles are also able to attach specifically to cell surface molecules that do not mediate cell entry and infection, but are used instead to present the virus to CD4-expressing T cells. This process, termed trans-infection, is very efficient and occurs when antigen-presenting dendritic cells (DC) encounter T cells [38]. HIV-1 can therefore bind to DC in the periphery, which transport the virus to lymphoid organs, where it is transferred to T cells that become infected. The C-type lectin DC-SIGN (DC-specific ICAM-3-grabbing nonintegrin) was the first HIV-1 receptor in DC to be characterized as a mediator of trans-infection [39]. DC-SIGN is specific for simple high-mannose glycans and fucose-containing glycosylations

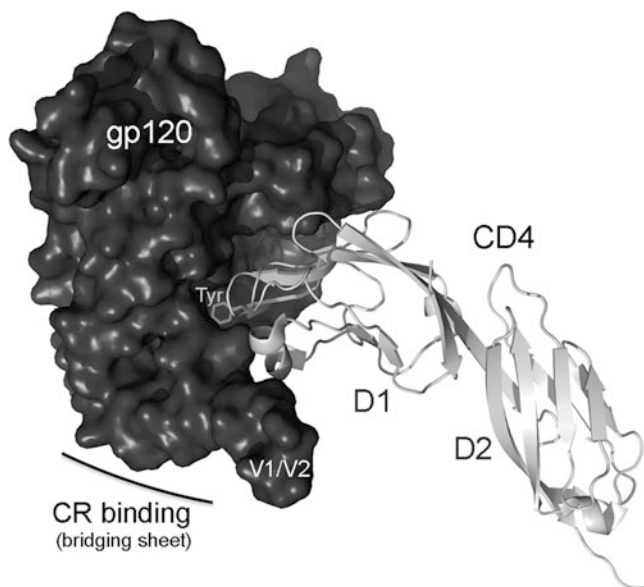


Fig. 15.4 HIV-1 receptor recognition. Crystal structure of the HIV-1 gp120 protein (*dark surface*) bound to the CD4 receptor (*ribbon drawing in grey*) (PDB ID 1GC1). The N-terminal domain of CD4 (D1) fits into a concave gp120 surface, with a deeply penetrating Tyr residue. The location is indicated of the truncated V1/V2 loop and the bridging sheet region that binds to HIV-1 entry co-receptors (CR). CR binding is necessary for virus-cell fusion at the plasma membrane. This and following Figs. 15.5 and 15.6 were prepared with PyMOL (pymol.org)

[40, 41]. gp120 is heavily glycosylated; its glycan composition is mostly of the high-mannose type, which is recognized by DC-SIGN. The glycan shield of the HIV-1 spike is thus used for attachment to DC that trans-infect T cells. It was recently shown that, in addition to the spike glycosylations, HIV-1 also uses charged glycans in its envelope lipidome to attach to DC [42, 43]. The HIV-1 envelope membrane is enriched in certain glycosphingolipids called gangliosides. A specific sialic acid-containing subset of gangliosides appears to recognize receptors on the DC surface [42, 43]. HIV-1 thus exploits different envelope components to attach to distinct cell types, which leads to spread of the infection.

Cell Receptor Recognition in Paramyxovirus

The paramyxoviruses are enveloped, negative-stranded RNA viruses that include serious human and animal pathogens [44]. In viruses of this family, cell attachment and virus-cell membrane fusion are mediated by two distinct membrane glycoproteins (see also Chap. 16). The paramyxovirus attachment proteins are type II membrane proteins anchored to the virus envelope by a single transmembrane domain [44, 45]. Their extracellular region can be divided into an N-terminal stalk region that serves as a spacer and a C-terminal globular domain with receptor binding activity. The C-terminal globular domains of paramyxovirus attachment

glycoproteins all fold into similar six-bladed β -propeller structures [44, 45]. In the virus envelope, the attachment proteins are present as disulphide-linked homodimers, with indications of tetramer formation in some cases [45]. The attachment proteins form complexes with the fusion proteins, which are homotrimers in the viral envelope. Receptor binding triggers rearrangements in these heterocomplexes that alter the structure of fusion proteins, resulting in fusion of viral and cell membranes at neutral pH [45–47] (see Chap. 16).

There is certain diversity in cell surface receptor usage among paramyxoviruses. Rubulaviruses (mumps virus), avulaviruses (Newcastle disease virus) and respiroviruses (Sendai virus) bind to cell surface sialic acids *via* the hemagglutinin neuraminidase (HN) attachment glycoprotein, a bifunctional protein engaged in recognition and hydrolysis of sialic acids. Neither activity is found in the haemagglutinin (H) of morbilliviruses (measles virus), or in the attachment glycoprotein G of henipaviruses (Hendra and Nipah viruses) or pneumoviruses (respiratory syncytial virus) [44]. Measles virus H (MV-H) and the Hendra (HeV-G) and Nipah G (NiV-G) proteins lack the conserved residues that mediate sialic acid binding and hydrolysis [5]. These viruses do not bind to sialic acid carbohydrates, but rather attach to cell surface proteins. HeV and NiV-G interact with the ephrin-B2 or -B3 receptors (EFNB2, EFNB3) [48–50], whereas MV-H can bind CD46 [51, 52], signalling lymphocytic activation molecule (SLAM) [53] or nectin-4 [54, 55], depending on the MV strain. All strains of MV bind to SLAM expressed on macrophages, DC, and lymphocytes, cells where infection starts and develops. MV uses nectin-4 to infect epithelial cells and cross the airway epithelium for transmission to new hosts [54, 55].

Structural studies defined the receptor binding modes of several paramyxoviruses. Both sialic acid and the EFNB2/EFNB3 receptors are recognized by overlapping sites at the recessed center of the β -propeller domain of the paramyxovirus attachment proteins (Fig. 15.5a) [44, 45, 56–58]. Protruding hydrophobic residues at the long GH loop of EFNB2 interact with residues in NiV-G that lie very close to the sialic acid binding site in HN at the central cavity of the β -propeller domain (Fig. 15.5a). The receptor binding mode in measles virus (MV) nonetheless differs from that of other paramyxoviruses. The structures of CD46 and SLAM in complex with MV-H protein show that MV uses the side of the β -propeller domain to bind to cell surface receptor molecules (Figs. 15.5a, b) [59, 60]. The recessed center of the β -propeller, the site of sialic acid binding in several paramyxoviruses, is closed off by a glycan in the MV-H protein. The receptor-binding region in MV-H includes a groove formed by the blades β_4 and β_5 in the β -propeller, a region with the largest structural difference between MV-H and the other paramyxovirus proteins [59]. Therefore, the receptor-binding regions in paramyxoviruses preserve the recessed nature shared by many receptor-binding sites in virus proteins (Figs. 15.5c, d). In addition, the receptor-binding regions contain a hydrophobic socket that is particularly recessed. This socket accommodates sialic acid in the paramyxovirus HN proteins or receptor-specific features in MV-H (Fig. 15.5e) [56, 59]. In the case of the MV-H, the recessed receptor-binding surface is more extended than in other paramyxovirus attachment protein using the centre of the β -propeller for attachment to cell surface receptors (Figs. 15.5c, d). This MV-H surface can recognise three distinct receptor molecules,

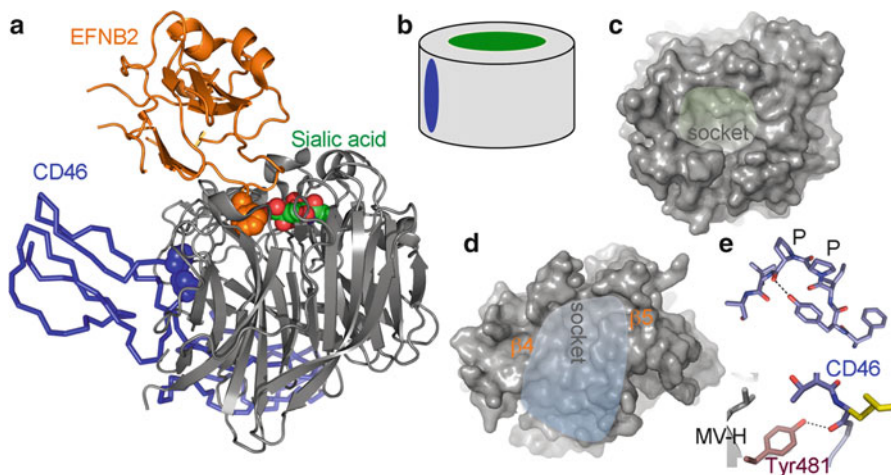


Fig. 15.5 Cell receptor recognition in paramyxovirus. (a) Ribbon drawing of the β -propeller domain of a representative paramyxovirus attachment protein (NDV-HN structure; grey), bound to three paramyxovirus receptors: Sialic acid (carbons (green) and oxygens (red) as spheres), the EFNB2 receptor (orange) and CD46 (blue). Two virus binding Pro residues in CD46 and one Phe in EFNB2 are shown as spheres. The figure was prepared with the crystal structures of complexes of NDV-HN with sialic acid (PDB ID 1E8T), of NiV-G with EFNB2 (PDB ID 2VSM), and of MV-H with CD46 (PDB ID 3INB) after superimposition of the virus proteins. NiV-G and MV-H structures are not shown. (b) Cylinder representing a β -propeller domain and location of main receptor binding surfaces in paramyxovirus attachment proteins, based on the complexes shown in (a); centre (green) and side (blue) of the propeller. (c) Top view of the NDV-HN structure showing the recessed sialic acid binding surface on the centre of the β -propeller (light green), with the socket in which the sialic acid binds (indicated). (d) Side view of the MV-H structure with the extended concave surface (light blue) use to bind to the SLAM, CD46 and nectin-4 receptors. The β -propeller blades β_4 and β_5 that form the receptor-binding surface and the socket on the surface are indicated. (e) Key interactions for MV recognition of CD46. *Top*. Stick diagram of protruding CD46 loop with two contiguous Pro residues that penetrates the MV-H socket. *Bottom*. Hydrogen bond (dashed black lines) of MV-H Tyr481 with a main chain carbonyl of CD46, required for MV binding to CD46 [59]. Oxygens are red and nitrogens dark blue

CD46, SLAM and nectin-4. As shown for the MV-H/CD46 complex [59], viral residues within the extended receptor-binding groove can show some variability, whereas less accessible residues are conserved; this leads to increasing binding affinity to alternative receptor molecules, which has an important implications on MV tropism and pathogenesis. Single residue mutations in the MV-H groove, such as the Asn481 to Tyr481 switch, is sufficient for MV binding to the ubiquitous CD46 receptor, which extends its cell tropism. Tyr481 can hydrogen bond to CD46 (Fig. 15.5e), an interaction responsible of the virus-receptor binding specificity. The use of an extended receptor-binding surface might explain MV recognition of multiple receptor molecules, which must be related to the efficient transmission of MV from host to host.

15.2.3 Carbohydrates as Viral Receptors

The cell surface displays a large variety of oligosaccharides linked to glycoproteins, proteoglycans and glycolipids, some of which are used by viruses to attach to host cells [61]. Most viral carbohydrate receptors are negatively charged and terminate the glycan moiety, features important for virus recognition. In some cases, however, viruses recognize neutral glycans such as histo-blood group antigens. Monomeric virus-carbohydrate interactions are usually of low affinity (mM range), however, virus particles have many recognition sites that easily engage several carbohydrate molecules, and thus attach to the cell surface with high avidity (Fig. 15.6a). Virus recognition of carbohydrates is associated also with functions other than cell attachment. Orthomyxo-, Paramyxo-, and Coronaviruses express envelope sialic acid-destroying glycoproteins (sialidases and esterases) that are essential for *in vivo* host infection. These enzymes can prevent re-attachment of newly released viruses from infected cells, can remove cell-bound viruses that fail to enter host cells, and can inhibit virus aggregation during budding. Sialidase inhibitors have proven to be useful anti-viral drugs for the treatment of some viral infections.

Sialic acid residues linked to glycoproteins and glycolipids act as receptors for many viruses. Sialic acids are derived from N-acetyl-neuraminic acid and mainly occupy the terminal position of a glycan chain, bound to a penultimate galactose through an (2–3) or (2–6) linkage [61], which renders them easily accessible. Moreover, sialic acids have a larger number of functional groups than other monosaccharides, and can thus participate in a network of polar and non-polar interactions with virus proteins (Fig. 15.6b) [62]. Sialic acids are receptors for distinct viruses, such as influenza, corona-, paramyxo-, toro-, adeno-, noro-, rota-, picorna-, parvo-, polyoma- and reovirus, some of which are important human pathogens [61]. Crystal structures of virus-sialic acid complexes showed that viruses use relatively recessed binding surfaces that interact mostly with the sialic acid face that bears a negatively charged carboxylate group (Figs. 15.6b, c, d) [62]. The mode of sialic acid recognition by distinct viruses can be similar, although viruses use structurally diverse proteins in receptor recognition. In some cases, however, related viruses such as murine polyomavirus and SV40 use similar capsid surface areas to recognize distinct motifs in the sialic acid molecules [5]. The virus-glycan contacts are thus not necessarily conserved (Fig. 15.6c, d).

Viral sialic acid receptors feature numerous cell-specific modifications that determine virus tropism, cell-to-cell transmission and pathogenicity. There are several examples of viruses responsible for serious diseases, such as adenovirus (Ad8, Ad19, Ad37) and enterovirus, which bind specifically to the (2–3)-linked sialic acid. The use of this sialic acid variant, which forms part of a branched glycan linked to the CD1a ganglioside, is responsible for the eye-tropism of those viruses, whose infection cause severe ocular diseases [29, 61]. Influenza A virus transmissibility and pathogenicity in humans also correlates with the recognition of specific sialic acid molecules by the envelope haemagglutinin (H) and neuraminidase (N)

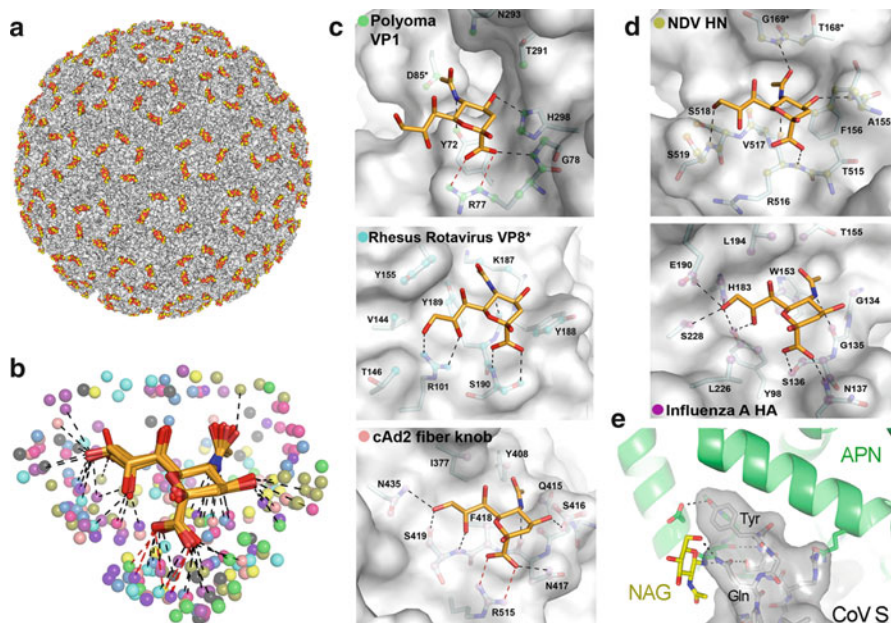


Fig. 15.6 Carbohydrates as viral receptors. (a) Multimeric binding of polyomavirus to its sialic acid receptor. Surface representation of the crystal structure (PDB ID 1SIE) of a polyomavirus particle (*surface in grey*) in complex with a sialic acid receptor fragment (*carbons in yellow*). (b) Possible contacts of viral proteins with terminal sialic acid, based on crystal structures [62]. Sialic acid is shown as sticks with carbons in orange. Viral atoms within 4.0 Å of the carbohydrate receptors in crystal structures of virus-sialic acid complexes are shown as spheres. Hydrogen bonds and salt bridges are indicated by *black* and *red* lines, respectively. (c) Non-enveloped viruses binding to sialic acid receptors. Detail of sialic acid binding interactions with polyoma VP1 (PDB ID 1VPS), rhesus rotavirus VP8* (PDB ID 1KQR) and cAd2 fibre knob (PDB ID 2WBV). (d) Enveloped viruses binding to sialic acid receptors. Network of interactions for the NDV-HN (PDB ID 1USR) and influenza HA protein (PDB ID 1HGG). (e) Coronavirus (CoV) binding to a glycan-containing region of its aminopeptidase N (APN) receptor. Detail of the interaction revealed by the crystal structure (PDB ID 4F5C) of a fragment of a porcine CoV spike protein (CoV S) in complex with the pig APN. CoV S is shown as *grey surface* and stick representations, the APN as *green ribbons*, with the key virus-binding N-acetyl-glucosamine (NAG) residue as sticks and *carbons in yellow*. The viral Tyr and Gln residues that contact the glycan in the CoV S protein are labelled. Hydrogen bonds are *dashed black lines*. Oxygens are *red* and nitrogens *dark blue* in all figures (Figures (b-d) were provided by U. Neu and T. Stehle, adapted with permission from [62])

proteins. The H (H1 to H15) and N (N1 to N9) protein variants identified in distinct species differ in their affinities for the (2–3)- or (2–6)-linked sialic acids, which is a key determinant in virus transmissibility [61]. Aquatic birds are the natural reservoir of influenza A, but the avian viruses are periodically transmitted to mammals, causing flu pandemics with significant numbers of deaths [63]. The H protein of avian influenza A (HA) binds preferentially to (2–3)-linked sialic acid receptors, which are not present in the human tracheal cells initially infected by influenza A [64];

transmission of avian influenza A from birds to humans or within the human population is therefore quite inefficient. Nonetheless, variants of some influenza A strains (H5N1) can infect humans, causing sporadic outbreaks with high mortality rates. Alterations in the virus receptor-binding specificity for (2–6)-linked sialic acids can cause the emergence of a pandemic such as the 1918 Spanish flu. In addition to these examples, other viruses can recognise specific substitutions in the sialic acid molecule [61].

Viruses can also bind to other negatively charged sulphated or neutral carbohydrates. Human herpesviruses are an example of viruses that attach to cell surface heparan sulphate (HS), and this interaction has been studied extensively for human herpes simplex viruses (HSV). HSV cell entry is complex and is mediated by several envelope glycoproteins (gC, gB, gD, gH and gL) [65]. The initial interaction of the virus particles with the cell is mediated by the gC and gB glycoproteins, which bind to HS. This interaction is nevertheless insufficient for virus entry, which also requires gD binding to cell surface proteins for virus-cell membrane fusion, catalysed by gB and the gH/gL complex. HS can thus be considered an HSV attachment factor that facilitates subsequent interactions with other receptors for cell entry.

Noroviruses exemplify a group of viruses that use neutral cell surface carbohydrate receptors, known as histo-blood group antigens [66]. Recognition is strain-specific and different virus strains recognize distinct types of antigens. These variations affect the tropism and pathogenesis of norovirus infections, as susceptibility to infection is dependent on the histo-blood group antigen receptors, which differ among individuals, who can therefore be resistant to infection by certain norovirus strains and susceptible to others. It is of interest to note that viruses can also recognize cell surface structures comprised by carbohydrates and amino acid residues, as recently described for some coronaviruses (CoV) [67]. A protruding loop with a tyrosine residue in the envelope spike of a subset of CoV docks between a neutral N-acetyl-glucosamine residue and an alpha helix exposed in the ectodomain of its receptor, the aminopeptidase N (APN) (Fig. 15.6e). This N-linked glycan is essential for virus binding to APN, and is a major determinant of the host range of some CoV [67].

Cell surface carbohydrates are ubiquitous, accessible and relatively variable “hooks” for cell attachment by highly distinct virus families. Carbohydrate-derived compounds can thus be used to inhibit many types of virus infections. Carbohydrates are major cell entry receptors for some viruses, and can be alternative or secondary entry receptors for others. Viruses that attach to protein receptors can evolve to use carbohydrates, thus expanding host cell tropism [4, 16, 68]. Enveloped viruses can also exploit the glycans linked to their envelope proteins to attach to cell surface lectins. Some of these lectins are specific for certain glycosylation patterns associated to virus membrane glycoproteins, as discussed above for DC-SIGN and HIV-1. Many enveloped viruses attach to host cells *via* DC-SIGN [6], a cell surface receptor for multiple pathogens. Glycans are thus intimately linked to virus-cell interaction.

15.3 Non-Enveloped Virus Entry into Host Cells: The Uncoating Process

15.3.1 Receptor-Mediated Uncoating of Picornaviruses

After binding to cell surface receptors, the viral genome must translocate from the particle to the cell cytoplasm (Fig. 15.1). In enveloped viruses, this transfer may be rather simple and occurs after fusion of the virus and cell membranes, as will be illustrated in Chap. 16. In non-enveloped viruses, mechanisms for genome penetration into the cytoplasm have not yet been characterized in detail, although several pieces of the process have been described. Particularly well-studied models include some viruses of the picornavirus family, PV and HRV, which will be discussed here.

The initial event during membrane penetration of picornaviruses is the opening of the viral capsid and the exit of the viral genome, a process known as uncoating. The capsids of PV and HRV are very similar and are characterized by a sedimentation coefficient of about 160S for PV and 150S for HRV; after RNA exit, the density of the empty capsids decreases markedly and their sedimentation coefficient is 80S [69–71]. Binding of soluble receptor molecules to the viral particles decreases their sedimentation coefficient to approximately 120S [72]. In addition, uncoating intermediates that lack the internal VP4 protein and have a sedimentation coefficient of about 135S have been described [69, 73, 74]. The distinct sedimentation behaviour of native virions and empty capsids allowed analysis of the uncoating process by ultracentrifugation in PV and HRV [70–72, 75, 76]. The structures of some of these capsid forms have been determined, and have helped to propose models for the structural rearrangements and dynamic processes associated with genome uncoating in these viruses (see below, Subsection 15.3.2).

The opening of the capsid in PV and HRV can be mediated by receptor binding, by low pH, or by the cooperative effect of both factors [71, 74–76]. Initial studies with PV and soluble poliovirus receptor (PVR) showed that receptor binding at physiological temperature (37 °C) mediates RNA exit from the capsid interior with no additional factors [70]. PVR binding to PV generates an intermediate particle with a sedimentation coefficient of 135S that lacks VP4, which is subsequently converted to empty 80S capsids. These particles are also observed during initial cell infection by PV [74], and thus represent entry intermediates. These findings show that PVR has an active role in PV entry; it is not just a “hook” used for attachment, but also an “unzipper” that mediates viral RNA uncoating [77]. Subsequent studies with HRV showed similar behaviour for the HRV receptor ICAM-1, which triggers RNA uncoating after binding to certain HRV serotypes at physiological temperatures [71, 72]. ICAM-1 binding to HRV serotypes 3 (HRV3) and 14 (HRV14) promotes RNA exit and formation of empty 80S capsids at neutral pH. This process is temperature dependent and requires temperatures over 25–30°C, necessary to overcome the high activation energy of the uncoating process (~45 kcal/mol) [72]; this energy is provided by receptor binding, an endothermic process in viruses sensitive to

receptor binding, such as PV and HRV3 [12]. PVR binding to PV is more endothermic than ICAM-1 binding to HRV3, which indicates that PVR is more efficient triggering uncoating than ICAM-1. As PV can enter host cells in the presence of agents that prevent endosomal acidification [74], RNA uncoating and transfer to the cytoplasm must therefore be mediated by receptor binding. PV infects hosts through the gastrointestinal tract; it is thus very stable at the low pH to which viruses are exposed during endocytosis. PV uses a receptor molecule suited to efficient uncoating at neutral pH and host cell entry.

The HRV are a group of viruses composed of around 100 different serotypes that bind to ICAM-1 (major group) or LDLR (minor group), as described above (Subsection 15.2.1). There is also certain diversity among serotypes in terms of the physiological factors that trigger HRV uncoating. Minor group HRV are sensitive to mildly acidic pH (5.5–6.0), which can trigger virus uncoating at physiological temperatures. Binding to LDLR does not mediate uncoating, and minor group HRV are thus dependent on endocytosis and endosome acidification for uncoating and host cell entry [16, 75]. The major group of HRV, which bind ICAM-1, comprises viruses that differ in stability and sensitivity to receptor binding [12, 71, 78]. Serotypes such as HRV3 are uncoated efficiently by ICAM-1 binding at neutral pH and physiological temperatures, whereas HRV16 are relatively stable and HRV14 have intermediate sensitivity. These differences in sensitivity to receptor binding are not related to affinity, but to binding energy. HRV3 binding to ICAM-1 is more endothermic than HRV16 binding [12]; the HRV3-receptor complex is thus less stable and is therefore primed for uncoating. On the contrary, HRV16-receptor complexes are more stable and require additional factors for uncoating, as explained below. In accordance with these findings, structural studies of HRV-receptor complexes prepared at physiological temperature show expansion of HRV3 following receptor binding and lack of expansion for HRV16 [79]. The energy absorbed by the HRV3-receptor interaction translates into capsid expansion, a metastable state primed for RNA exit.

The low pH at which cell surface receptor-bound viruses are exposed during endocytosis is a relevant factor that mediates virus uncoating (Fig. 15.1) [3]. Indeed, the mildly acidic pH (5.5–6.0) in endosomal compartments is optimal for uncoating of and infection by minor group HRV [16, 75, 80]. Exposure to low pH is also necessary for efficient uncoating and cell entry by some major group HRV [16, 76], although certain serotypes require only ICAM-1 binding, after which they can infect cells, even in the presence of agents that prevent endosome acidification. In the case of relatively stable HRV serotypes with pocket factor molecules, such as HRV16, both receptor binding and low endosome pH are needed for entry into host cells. The uncoating rate of HRV16 after binding ICAM-1 or exposure to mildly acidic pH (5.5–6.0) is very slow, and the virus can remain intact for several hours [71, 76]. After binding to ICAM-1, however, HRV16 particles become sensitive to low pH, which triggers rapid RNA release from the capsid [76]. Receptor binding appears to alter the HRV16 particles, priming them for low pH-mediated uncoating during endocytosis. This cooperative effect of the receptor and low pH in virus uncoating must be necessary to polarize RNA exit at the receptor-bound region of

the virus particle in endosomes, which must be relevant for efficient penetration of the cell membrane and infection.

The diverse receptor-mediated uncoating observed among major group rhinoviruses has been linked to the inherent stability and dynamics of the capsid. Biochemical studies of HRV indicate dynamic capsid behaviour [78, 81]; internal capsid polypeptides in the crystal structures, such as VP4 and the N terminus of VP1, are very sensitive to proteases, indicating that they are exposed to the environment at physiological temperatures. The dynamic behaviour of the capsids was termed “breathing” (see Chap. 6), and is likely to be mediated by the uncoordinated expansive-contractile movement of capsid protomers. Capsid breathing in HRV appears to be restricted by small molecules bound to a pocket in the VP1 [78, 81]. Natural molecules or pocket factors were identified as fatty acids in the capsids of the major group HRV16 and minor group HRV2. A group of molecules known as WIN compounds also bind to the VP1 pocket, restricting capsid dynamics and preventing uncoating. Moreover, the receptor-sensitive HRV3 serotype lacks pocket factor, which is present in the receptor-resistant HRV serotypes HRV16 and HRV2. These data suggest that capsid breathing is necessary for triggering receptor-mediated uncoating at physiological temperatures [79, 82].

15.3.2 The Structural Bases of Receptor-Mediated Virus Uncoating in Picornaviruses

Several structural studies over the last decade have examined the uncoating of PV and HRV intermediate particles by cryo-EM. One analysis determined the structure of the 135S PV particles, which lack VP4 but preserve the RNA inside, and compared this structure with 160S native virion and 80S empty capsids formed after RNA exit [73]. The 135S particle showed VP rearrangements relative to the native particle and 4 % expansion. Major rearrangements appear in the two-fold axes of the capsid and in the canyon region; however, the conformation of the five-fold axis, which was considered the RNA exit port, was similar to that of the 160S native PV capsid. HRV3 and ICAM-1 complexes prepared at 37 °C and in the process of uncoating were analysed by cryo-EM [79]; the structure also showed HRV3 capsid expansion not seen in stable HRV16-ICAM-1 complexes, indicating that capsid expansion must precede uncoating. The conformation of the RNA-containing HRV3 capsid in the virus-receptor complexes is similar to that of the native HRV capsid. Nevertheless, the empty HRV3 capsid is quite different, showing major rearrangements after RNA release; this has now been described in detail in a high-resolution structural study of empty HRV capsids [83]. Major differences between full and empty capsids in the HRV-receptor complexes appear at the five-fold axes; however, the structure of the five-fold axis regions are almost identical to those of native viruses in RNA-containing HRV3-receptor complexes [79]. These structural insights challenged the hypothesis of RNA exit through the

capsid five-fold axis, suggesting other exit ports. Recent studies with PV showed that RNA exit from the capsid near a two-fold axis, at a site that extends toward the receptor-binding canyon region [84].

The studies discussed here for PV and HRV show that receptors participate in the virus entry process, mediating not only virus-cell attachment but also RNA release, alone or with the contribution of low pH. The uncoating process requires multivalent receptor binding and capsid dynamics, which must explain the temperature dependence of this process. At physiological temperatures, capsids are dynamic entities whose protomers can move randomly, opening and closing holes at interprotomer junctions. This rapid, random movement does not allow for RNA release, probably because of the transient nature of the holes. After receptor binding at physiological temperatures, the capsid remains expanded and holes remain open for RNA release. PV and HRV recognize receptors *via* the canyon, a fragmented region at the junction of two protomers. PVR and ICAM-1 receptors can therefore act as wedges by binding at protomer junctions to maintain capsid expansion [12, 79], thus catalysing the uncoating process by locking the capsid in an intermediate, open state that allows RNA exit. The function of these two receptor molecules in virus uncoating is probably closely linked to the way they are recognized by PV and HRV.

The externalized genome in non-enveloped viruses either on the cell surface or in endosomes must translocate into the cell cytoplasm to initiate the intracellular phase of the life cycle. The study of the transfer of the genome from the virus particle to the cytoplasm is less understood in non-enveloped than in enveloped viruses. Structures of isolated envelope fusion proteins have delineated the process of membrane penetration or fusion of virus-cell membranes, described in Chap. 16. However, the analysis of viral genome penetration process in non-enveloped viruses requires the study of virus infecting host cells, which is methodologically challenging. Research carried out to date indicates two possible routes of penetration, either by membrane rupture or pores through which genome moves to the cytoplasm [16]. These disruptions in the membrane can be caused by hydrophobic capsid motif exposed during the uncoating process. The PV and HRV particles discussed here externalise the hydrophobic N-terminal region of VP1 that enable attachment of the particles to cell membranes in endosomes or on the cell surface. Moreover, the VP4 protein that is externalized together with the RNA bears a myristoyl group that can also interact with the cell membrane. The interaction of capsid motifs with the membrane can mediate its disruption for the transfer of the uncoated genome to the cytoplasm.

15.4 Perspectives and Conclusions

Viruses subvert cell surface molecules for cell entry and dissemination of infection. Receptor accessibility and cell expression patterns are important factors that underlie virus selection of specific surface receptors. Most of these receptors promote translocation of the viral genome into the cell. In some cases, however, viruses

attach to cellular factors that concentrate infectious particles on the plasma membrane and facilitate virus recognition of entry receptors. In other cases, viruses use cell surface molecules to spread the infection throughout the body; they can attach to migratory cells or induce signalling events that loosen cell-cell contacts and facilitate virus transmission. Cell surface molecules can thus have diverse functions in the dissemination of viral infections; the study of virus-receptor interactions is therefore of great relevance for understanding virus evolution, host tropism and pathogenesis.

The virus entry receptors have been the main focus of this chapter. These molecules are not just a “hook” for virus attachment, but can also participate in translocation of the viral genome into host cells, on the cell surface, or within endosomal compartments. Some virus receptors catalyse the entry process alone or in combination with other cell factors, such as the mildly acidic pH found in endosomes. Interaction with certain receptors transforms virus particles into metastable entities, priming delivery of their genomes to the cytoplasm. Alternatively, genome translocation can be mediated by low pH, but this requires endocytosis of receptor-bound viruses. The process of receptor-mediated virus entry continues to be studied. In some picornaviruses, discussed here, the energy absorbed in the virus-receptor interaction translates into virus particle expansion and genome exit. In other non-enveloped viruses, capsid expansion triggered by cell factors might be a necessary intermediate step for genome uncoating. Receptor binding also primes virus-cell membrane fusion in enveloped viruses, mediated by conformational changes in virus fusion proteins (Chap. 16). Cell surface molecules are thus key players in virus entry into host cells.

Virus-receptor interactions appear to be highly specific. There are many examples of virus selection of only a single member of closely-related cell surface molecules. This specificity is based on the recognition of certain unique structural features of a cell surface molecule, as well as on key polar interactions such as those described here for MV and its CD46 receptor. Virus-receptor surface complementarity must be important for virus recognition of certain receptor molecules. Viral proteins are shaped to dock into structural features of their receptors. Many viruses use concave surfaces for binding to cell surface receptors, which optimises receptor contact area and hides receptor-binding residues from antibodies. Nevertheless, viruses can also have protruding receptor-binding surfaces for receptor recognition. In both cases, viruses can escape from antibody neutralisation by mutating non-essential residues in these receptor-binding surfaces. Immune system pressure on viruses is a major determinant for the switch in receptor recognition observed in many virus groups, although this diversity can also be linked to opportunities to spread infection using ubiquitous or more accessible cell surface molecules. In addition to its high degree of specificity, virus-receptor recognition can be dynamic, and viruses can evolve to use alternative or distinct receptors, as illustrated here with several examples.

Preventing virus binding to receptors can efficiently block virus infection and cell damage. Receptor binding regions in virus proteins are relatively invariant and could potentially be targeted by immune responses to prevent infection. A large

number of structural studies have characterised viral antigenic sites targeted by neutralising antibodies. Many of these sites overlap receptor-binding regions, showing that blockade of virus binding to cell surface receptors is a major neutralisation mechanism. In viruses for which efficient vaccines have been developed (MV and PV), the receptor-binding residues are relatively well-exposed and accessible to antibodies. Viruses have nonetheless developed ways to evade neutralisation by hiding receptor-binding regions from antibodies; the use of concave and poorly accessible receptor-binding surfaces is one well-defined strategy of this type. In some viruses, these relatively inaccessible surfaces are also surrounded by variable loops and glycans, which further prevent antibody binding and neutralisation. Blocking infection of these viruses requires the design of improved vaccines that elicit immune responses focused on relatively inaccessible sites. These therapies require a deep understanding of virus-receptor interactions, including the determination of complex structures. These studies also open avenues for the development of molecules that prevent virus entry into cells. Soluble multimeric receptor molecules that impede virus binding to cells and infection were one of the first therapeutics developed for viruses such as HIV or HRV. Small molecule drugs have been developed, such as sialic acid analogues to treat influenza virus infection, or molecules that prevent HIV gp120 binding to its receptor, CD4 (see Chap. 20). The characterization of virus-receptor interactions is thus of considerable interest for the development of antiviral therapies.

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Further Reading

Especially recommended for further reading are the following references listed above: References [1–3] present a general overview of virus-receptor recognition and virus entry into host cells; references [4, 5] describe virus switch of receptor specificity; reference [6] is part of a *Current Opinion in Virology* issue describing recent research on virus entry. I also recommend the following references related to specific chapter sections: 15.2.1, references [8, 16] (picornavirus), and [22] (adenovirus); 15.2.2, references [34, 35] (HIV-1), and [45, 46] (paramyxovirus); 15.2.3, references [61, 62]; 15.3, references [16, 74, 79].