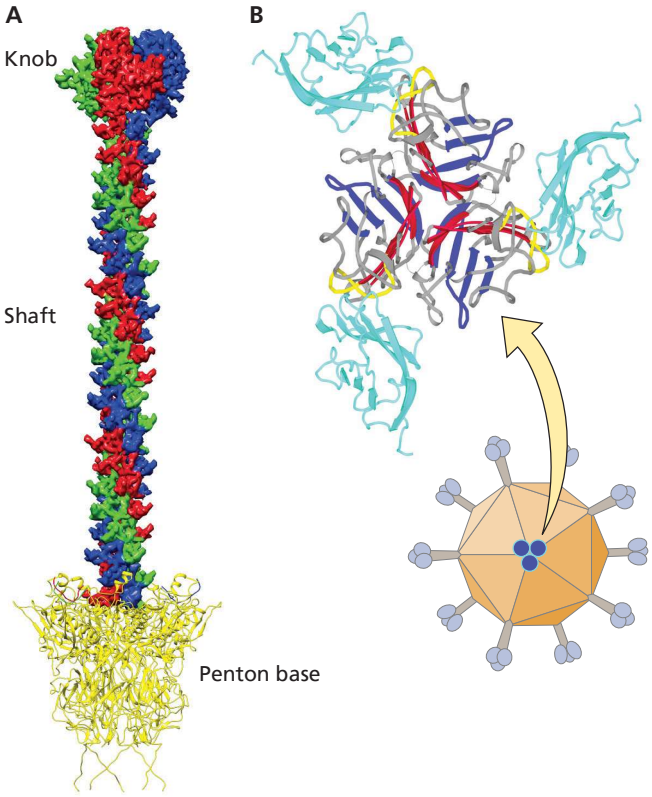


**Figure 5.5 Structure of the adenovirus 12 knob bound to the CAR receptor. (A)** Structure of a fiber protein, with knob, shaft, and tail domains labeled. Figure provided by Hong Zhou, University of California, Los Angeles, and Hongrong Liu, Hunan Normal University. **(B)** Ribbon diagram of the knob-CAR complex as viewed down the axis of the viral fiber. The trimeric knob is in the center (red-blue). The AB loop (yellow) of the knob protein contacts the first Ig-like domains of CAR molecules (cyan). The binding sites of both molecules require trimer formation.

uent shaft regions appear to form a rigid triple-helical structure in the trimeric fiber. The C-terminal 180 amino acids of each subunit interact to form a terminal knob. Genetic analyses and competition experiments indicate that determinants for the initial, specific attachment to host cell receptors reside in this knob. The structure of this domain bound to CAR reveals that surface loops of the knob contact one face of the receptor (Fig. 5.5B). Attachment to integrins is mediated by amino acid sequences in each of the five subunits of the adenovirus penton base that mimic the normal ligands of these molecules.

**Glycolipid receptors for polyomaviruses.** The family *Polyomaviridae* includes simian virus 40, mouse polyomavirus, and human polyomavirus 1. Members of this family are unusual because they bind to gangliosides, which are glycosphingolipids with one or more sialic acids linked to a sugar chain. There are more than 40 known gangliosides, which differ in the position and number of sialic acid residues critical for virus binding. Simian virus 40, polyomavirus, and human polyomavirus 1 bind to three different types of ganglioside. Structural studies have revealed that sialic acid linked to galactose by an α(2,3) linkage binds to a pocket on the surface of the polyomavirus capsid. Gangliosides are highly concentrated in lipid rafts (Chapter 2, Box 2.1) and participate in signal transduction, two properties that are important during polyomavirus entry into cells.

Gangliosides are the only surface molecule identified, to date, to be required for human polyomavirus 1 entry. In contrast, after binding a ganglioside, mouse polyomavirus interacts with $\alpha_4\beta_1$ integrin to allow virus entry. Furthermore, gangliosides are not the primary receptor for simian virus 40. This virus first binds to major histocompatibility class I (MHC-I) molecules on the cell surface and the complex migrates to caveolin-rich membrane domains that are also enriched in gangliosides and mediate endocytosis (Fig. 5.6). In contrast, another polyomavirus, human polyomavirus 2, relies not on gangliosides and caveolin pit-mediated endocytosis but on the serotonergic receptor $5HT_{2A}R$ and ligand-induced clathrin-mediated endocytosis.

### *Alternative Attachment Strategies*

The examples provided above highlight the diversity of cell surface molecules that can serve as viral receptors and demonstrate that entry of many viruses requires more than one cell surface molecule. In contrast, some nonenveloped virus particles bind to different cell receptors, depending on the nature of the virus isolate or the cell line. Often passage of viruses in cells in culture selects variants that bind heparan sulfate. Infection of cells with foot-and-mouth disease virus type A12 requires integrin $\alpha_v\beta_3$. However, the receptor for the O strain of this virus, which has been extensively passaged in cells in culture, is not integrin $\alpha_v\beta_3$ but cell surface heparan sulfate. On the other hand, the type A12 strain cannot infect cells that lack integrin $\alpha_v\beta_3$, even if heparan sulfate is present.

### *Transmembrane Glycoproteins of Enveloped Viruses Mediate Attachment and Entry*

The lipid membranes of enveloped viruses originate from those of the host cells. Membrane-spanning viral proteins are inserted into these cellular membranes by the same mechanisms as cellular integral membrane proteins and incorporated in the budding virus particles. Attachment sites on one or more of these envelope proteins bind to specific receptors. The two best-studied examples of enveloped virus attachment and its consequences are provided by the interactions of the envelope proteins of influenza A virus and the human immunodeficiency virus type 1 with their receptors.

**Influenza virus.** The family *Orthomyxoviridae* comprises the three genera of influenza viruses, A, B, and C. These viruses bind to negatively charged, terminal sialic acid moieties present in oligosaccharide chains that are covalently attached to cell surface glycoproteins or glycolipids. The presence of

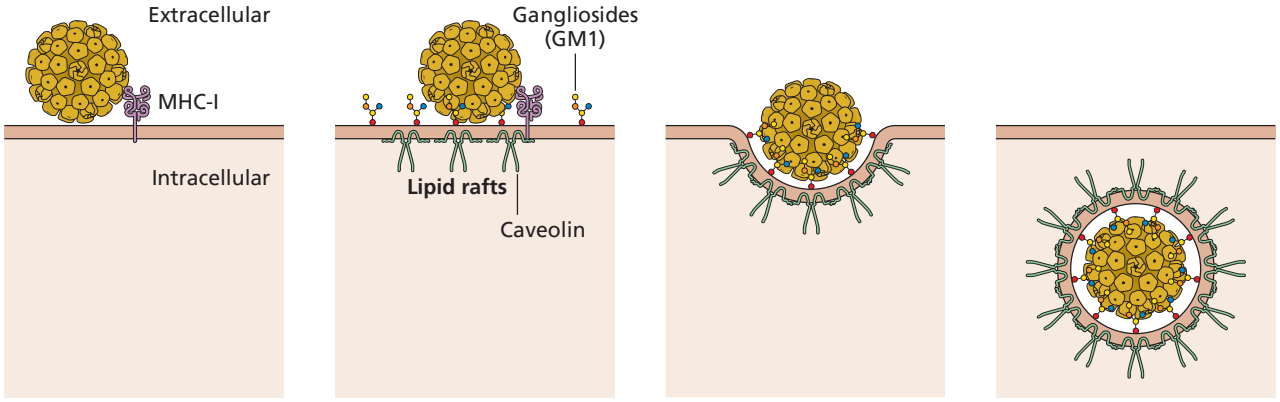


**Figure 5.6 Entry of polyomavirus simian virus 40.** Simian virus 40 interacts first with MHC class I molecules and bound virus particles move into small pits, 60 to 80 nm in diameter, rich in caveolin, known as caveolae. These pits are enriched in gangliosides and cholesterol. Several polyomaviruses bind to gangliosides with a terminal sialic acid linked by an α(2,3) bond to the penultimate galactose. Binding of virus particles to gangliosides induces intracellular signaling and membrane curvature that result in formation of caveolae that are endocytosed, bringing the virus particles inside the cell.

sialic acid on most cell surfaces accounts for the ability of influenza virus particles to attach to many types of cells. The interaction of influenza virus with individual sialic acid moieties is of low affinity. However, the opportunity for multiple interactions among the numerous attachment proteins on the surface of the virus particle and multiple sialic acid residues on cellular glycoproteins and glycolipids results in a high overall avidity of the virus particle for the cell surface.

The virus glycoprotein that binds to the cell receptor sialic acid is HA (hemagglutinin). The HA trimer is synthesized as a precursor that is glycosylated, and subsequently each monomer is cleaved to form HA1 and HA2 subunits that remain attached via disulfide bonds. Each HA monomer consists of a long, helical stalk anchored in the membrane by HA2 and topped by a large HA1 globule, which includes the sialic acid-binding pocket (Fig. 5.7A). While attachment of all influenza

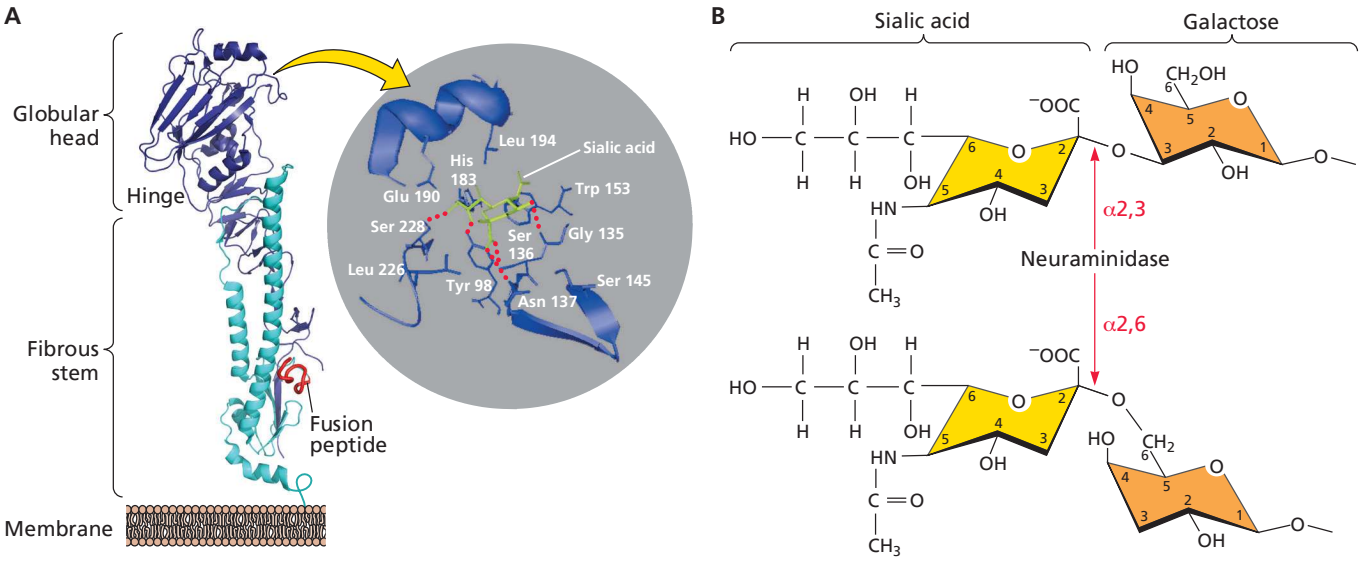


**Figure 5.7 Interaction of sialic acid receptors with the hemagglutinin of influenza viruses. (A)** Structure of the cleaved ectodomain of a monomer of the HA1-HA2 glycoprotein (based on X-ray crystallography data; PDB ID: 4FNK). HA1 (blue) and HA2 (cyan) subunits are held together by a disulfide bridge as well as by many noncovalent interactions. The fusion peptide at the N terminus of HA2 is indicated (red). (Inset) Close-up of the receptor-binding site with a bound sialic acid molecule. Side chains of the conserved amino acids that form the site and hydrogen-bond with the receptor are included. **(B)** The structure of a terminal sialic acid moiety that is recognized by HA. Sialic acid is attached to galactose by an α(2,3) (top) or an α(2,6) (bottom) linkage. The site of cleavage by the influenza virus envelope glycoprotein neuraminidase is indicated. The sialic acid shown is *N*-acetylneuraminic acid, which is the preferred receptor for influenza A and B viruses.

A virus strains requires sialic acid, strains vary in their affinities for different sialyloligosaccharides. For example, human virus strains bind preferentially sialic acids attached to galactose via an α(2,6) linkage, the major sialic acid present on human respiratory epithelium (Fig. 5.7B). Avian virus strains bind preferentially to sialic acids attached to galactose via an α(2,3) linkage, the major sialic acid in the duck gut epithelium. Amino acids in the sialic acid-binding pocket of HA (Fig. 5.7A, inset) determine which sialic acid is preferred and can therefore influence viral host range. It is thought that an amino acid change in the sialic acid-binding pocket of the 1918 influenza virus, which may have evolved from an avian virus, allowed it to recognize the α(2,6)-linked sialic acids that predominate in human cells.

The surfaces of influenza viruses were shown in the early 1940s also to carry an enzyme that, paradoxically, removes the receptors for attachment from the surface of cells. Later, this enzyme was identified as the virus-encoded envelope glycoprotein neuraminidase, which cleaves the glycoside linkages of sialic acids (Fig. 5.7B). Although the presence on virus particles of an enzyme that cleaves the virus receptor might appear puzzling, this activity is required not for entry but rather for efficient release of newly produced virus particles. Once such particles are synthesized and bud from the cell surface, the neuraminidase cleaves the sialic acid on the surface of these infected cells to free trapped particles, facilitating virus spread through the respiratory tract (Chapter 13).

**Human immunodeficiency virus type 1.** The acquired immunodeficiency syndrome (AIDS) pandemic has focused great attention on its causative agent, the lentivirus human immunodeficiency virus type 1. When examined by electron microscopy, the envelopes of human immunodeficiency virus type 1 and other retroviruses appear to be studded with "spikes" (see Fig. 4.18). These structures are composed of trimers of the single viral envelope glycoprotein, which mediates receptor binding and fusion. The monomers of the spike protein are synthesized as heavily glycosylated precursors that are cleaved by a cellular protease to form SU and TM (Fig. 5.8A). The latter is anchored in the envelope by a single membrane-spanning domain and remains bound to SU by numerous noncovalent bonds.

The primary cell receptor for human immunodeficiency virus type 1 is CD4, a 55-kDa rod-like protein that is a member of the Ig superfamily and has four Ig-like domains (Fig. 5.3). A variety of techniques have been used to identify the site of interaction with human immunodeficiency virus type 1, including site-directed mutagenesis and X-ray crystallographic studies of CD4 bound to the viral attachment subunit SU (Fig. 5.8B). The CD4-binding site in SU is a deep cavity, and the opening of this cavity is occupied by CD4 amino acid Phe43, which is critical for SU binding. This Phe43 is in a region analogous to a Phe127 in CD155 that binds to poliovirus. Remarkably, two viruses with entirely different architectures bind to analogous surfaces of these Ig-like domains. Comparison of the structure of SU in the presence and absence of CD4 indicates that receptor binding induces conformational changes in SU. These changes expose binding sites on SU for the chemokine receptors, which are required for fusion of viral and cellular membranes (see Box 5.1 and "Membrane Fusion" below).

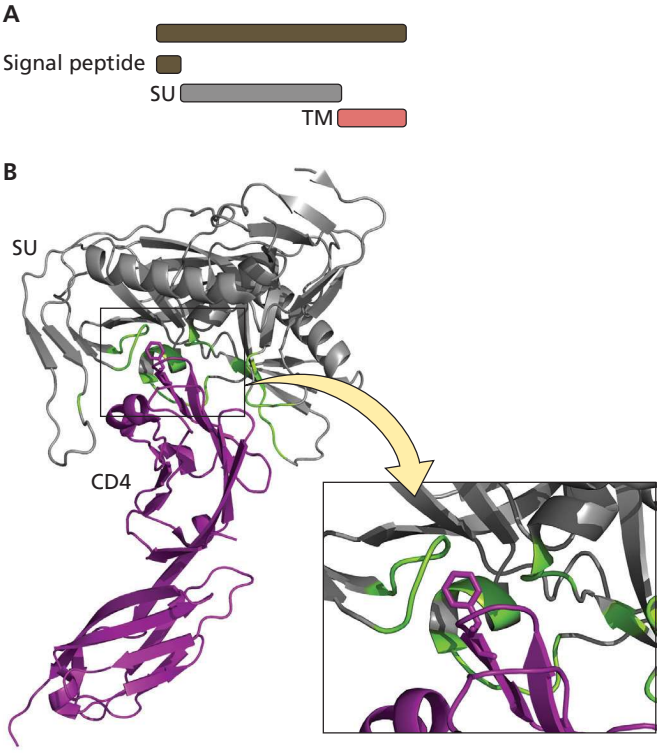


**Figure 5.8 Interaction of human immunodeficiency virus type 1 envelope glycoprotein with its receptor. (A)** The HIV-1 envelope precursor is cleaved to produce two subunits, SU (surface) and TM (transmembrane), that remain noncovalently attached. **(B)** Ribbon diagram of an SU monomer (gray) bound to CD4 (purple) (based on X-ray crystallographic data; PDB ID: 2NY1). SU residues that form the CD4-binding site are colored green. The side chain of CD4 Phe43, a residue critical for binding to SU, is shown penetrating the hydrophobic cavity of SU. This amino acid, which makes 23% of the interatomic contacts between CD4 and SU, is at the center of the interface and appears to stabilize the entire complex.

**Virus particles with multiple attachment and entry proteins.** Entry appears far more complicated in the case of herpesviruses, which have multiple surface glycoproteins that coordinate in different ways to guide entry via distinct routes depending on the target cell. The components affecting selection of particular routes of entry are not well understood. For example, herpes simplex virus 1 particles have 12 glycoproteins