

How Viruses Enter Animal Cells

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Viruses replicate within living cells and use the cellular machinery for the synthesis of their genome and other components. To gain access, they have evolved a variety of elegant mechanisms to deliver their genes and accessory proteins into the host cell. **Many animal viruses take advantage of endocytic pathways and rely on the cell to guide them through a complex entry and uncoating program.** In the dialogue between the cell and the intruder, the cell provides critical cues that allow the virus to undergo molecular transformations that lead to successful internalization, intracellular transport, and uncoating.

Although extremely simple in structure and composition, viruses are masters of camouflage and deception. Devoid of any means of independent locomotion, they disseminate by exploiting cells and organisms. Aided by rodents, insects, and migratory birds, and passed along by global trade and travel, they move around the world with amazing speed. Once they enter the body of a potential host, they can penetrate mucus layers, move through the blood stream, and disperse with the help of motile cells and neuronal pathways.

A critical moment occurs when a virus particle reaches a potential host cell and attaches itself to the surface. It must now deliver its capsid and accessory proteins into the cell in a replication-competent form, ideally with minimal damage to the cell and leaving little evidence of its entry for detection by the immune defenses. This is not a trivial problem because cell membranes are impermeable to macromolecules.

Overview: Virus Entry and Uncoating

Viral particles mediate the transfer of the viral genome and accessory proteins from an infected host cell to a noninfected host cell. The task involves packaging the viral genome (RNA or DNA) and accessory proteins, releasing the package from the infected cell, protecting the essential components during extracellular transmission, and delivering them into a new host cell. Many viruses with a DNA genome must enter the nucleus, whereas RNA viruses, with a few exceptions, replicate in the cytosol. Overall, viruses use a “Trojan horse” strategy in which the victim assists the intruder. To extract assistance from the host cell, viruses use the detailed “insider information” that they have acquired during millions of years of coevolution with their hosts.

In a typical animal virus particle, the viral RNA or DNA is condensed in icosahedral or

helical nucleoprotein complexes called capsids. In enveloped viruses, the capsids are surrounded by a lipid bilayer that contains viral spike glycoproteins. In addition, some viruses contain reverse transcriptases, RNA polymerases, kinases, and other proteins that are important during uncoating, replication, or other early intracellular steps.

To infect a target cell, a virus particle proceeds through a multistep entry process, during which each step is preprogrammed and tightly regulated in time and space. Figure 1 shows electron micrographs of some entry steps: virus binding to the cell, endocytosis, and nuclear import. Another critical step in the infection process is uncoating, during which the lipid envelope must be shed and the capsids must be at least partially disassembled to expose a replication-competent genome. Once uncoating has occurred, the mobility of the genome within the cell is restricted.

Progress through the entry and uncoating program depends on “cues” that the cell provides. Cues include interaction with cell surface receptors, exposure to low pH, and reimmersion into a reducing environment. Such cues trigger preprogrammed conformational changes and dissociation events in the virus particle.

To respond to cues, the virus particles or some of their component proteins (such as the spike glycoproteins) occur in metastable and easily modified conformational states. When triggered by a cue, the metastable state can be relaxed to allow marked changes in viral properties without the input of external energy. Here, we describe several examples of this process.

Receptors and Attachment Factors

To infect, a virus must first attach itself to the surface of a cell. The molecules to which viruses bind constitute a diverse collection of cellular proteins, carbohydrates, and lipids. They differ from one virus to the next, and they range from abundant and ubiquitous to rare and cell specific. Some of them merely serve as attachment factors that concentrate viruses on the cell's surface. Others are true receptors in that they not only bind viruses

but are also responsible for guiding the bound viruses into endocytic pathways and for transmitting signals to the cytoplasm. Receptors can also serve as cues that induce conformational changes that lead to membrane fusion and penetration. The identity and distribution of attachment factors and receptors determines to a large extent which cell types, tissues, and organisms a virus can infect.

Some viruses use multiple attachment factors and receptors in parallel or in succession. In the case of human immunodeficiency virus (HIV)–1, for example, initial contacts often involve cell surface attachment factors such as mannose binding C-type lectin receptor family members, the dendritic cell-specific intercellular adhesion molecule (ICAM)–3–grabbing nonintegrin (DC-SIGN), or the liver and lymph node-specific ICAM–3–grabbing nonintegrin (L-SIGN) (1, 2). These initial interactions do not induce conformational changes in the glycoprotein. When the glycoprotein 120 (gp120) subunit of the virus envelope binds to the outermost immunoglobulin G domain of CD4, it undergoes a conformational change that allows the virus to associate with its co-receptors, the chemokine receptors CXCR4 or CCR5 (3). The interaction between gp120 and CXCR4 or CCR5 triggers the conversion of the gp41 envelope subunit to the fusion-competent conformation (4, 5).

A similar situation is observed for alpha-herpes viruses, for which heparan sulfate proteoglycans serve as initial attachment factors for one of the glycoproteins (gC). Membrane fusion is induced by other viral glycoproteins after interacting with additional receptors, such as herpes virus entry (HVE) mediator, nectins, or integrins (6).

The individual interaction between a virus and a single attachment factor or receptor can be weak with a binding constant as low as millimolar (7, 8). However, when multiplied over numerous contacts, the avidity makes virus binding virtually irreversible. Enveloped viruses such as myxo- and paramyxoviruses that bind to sialic acid groups have spike glycoproteins with neuraminidase activity. They serve as receptor-destroying factors that release the bound viruses if these viral particles fail to proceed in their entry program (9).

Receptor-Binding Proteins

In enveloped viruses, it is the spike glycoproteins that bind to receptors. They are often multifunctional proteins serving additionally as membrane fusion factors and/or receptor-destroying enzymes. Structural data on spike

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glycoprotein–receptor interactions exist for several enveloped viruses including hemagglutinin (HA) of influenza virus with bound sialic acid (7), for gp120 of HIV-1 with bound CD4 (10), for glycoprotein D of herpes simplex virus 1 (HSV-1) with bound HVEA (11), for gp42 of Epstein-Barr virus with bound human lymphocyte antigen (HLA)–DR (12), and for Newcastle disease virus HN protein with the beta-anomer of sialic acid (13).

In non-enveloped viruses, the structures that bind receptors are projections or indentations in the capsid surface. Adenoviruses have prominent homotrimeric fibers with globular knobs that project from each of the 12 vertices. The x-ray crystal structure of the adenovirus 12 knob, together with the N-terminal domain of the coxsackie and adenovirus receptor (CAR), shows a large contact area on the lateral side of each subunit in the knob (14). The penton base of many adenovirus subfamilies contains an exposed RGD sequence that associates with integrins (15). In rhino- and enteroviruses, including polio, the receptors bind in a cleft in the capsid surface called the “canyon” (16).

For some viruses, binding may cause destabilization of the virus particle, a first step toward uncoating.

Virus Binding: Carbohydrate/Protein Interactions

Carbohydrate/protein interactions have long been known to play an important role in viral invasion (17). Some viruses bind specifically to sialic acid-containing groups, and others bind to glycosaminoglycans or glycolipids. Heparan sulfate has been identified as an attachment factor for herpes viruses, adeno-associated viruses, dengue virus, tick-borne encephalitis virus, papilloma viruses, paramyxovirus 3, and Sindbis virus (6, 18–23). For some of these, the degree of sulfation of the heparan sulfate or the presence of groups generated by specific sulfotransferases is important (6, 24). In most cases, carbohydrates serve as attachment factors that do not trigger conformational changes.

Simian virus 40 (SV40) and polyoma virus use gangliosides (GM1, GD1a, and GT1b) during attachment (25, 26). In polyoma virus, the disaccharide sialic acid– α 2,3-galactose present in these gangliosides binds to a shallow pocket in the major capsid protein, VP1 (8).

In some virus systems, the lectin is located on the cell surface and the carbohydrate ligand is located on the virus. HIV-1, Sindbis virus, Dengue virus, human cytomegalovirus, hepatitis C virus, and Ebola virus all bind to cell surface lectins, such as DC-SIGN and L-SIGN, through high mannose N-linked glycans in their envelope glycoproteins (27–32).

Endocytosis

Many animal viruses rely on the cell's endocytic machinery for productive infection.

Figure 2 shows schematically the endocytic entry pathways of three viruses that replicate in the nucleus: adenovirus 2, influenza A, and SV40.

One of the advantages of endocytic entry is that viruses are given a “free ride” deep into the cytoplasm. This is because endo-

cytic vesicles are designed to traverse the barriers imposed by the cortical cytoskeleton and the highly structured cytoplasm. Depending on the virus, incoming virus particles can be seen entering endosomal structures, lysosomes, the endoplasmic reticulum (ER), and occasionally the Golgi complex (33, 34).

A further advantage of endocytosis is that incoming viruses are exposed to compartmental environments that differ from the extracellular milieu. For many viruses, the mildly acidic pH in endosomes provides an essential cue that triggers penetration and uncoating (35–37). Penetration from intracellular vacuoles also has the advantage of leaving no viral glycoproteins exposed on the cell surface for immune detection. Finally, if penetration is lytic—as is the case for adenoviruses—endosomal membrane lysis is likely to be less damaging to the cell than lysis of the plasma membrane.

One risk during endocytosis is possible delivery to the lysosome, a degradative compartment and a dead-end for most viruses. This is why viruses have carefully adjusted the threshold pH for activation to match that of early (pH 6 to 6.5) or late endosomes (pH 5 to 6) (38, 39). That early and late endosomes constitute distinct entry sites has recently been confirmed with dominant negative mutants of endosome-associated small guanosine triphosphatases (GTPases) (40). A constitutively inactive mutant of rab5 (early endosome) blocked the entry of both Semliki Forest virus (pH 6.2) and influenza virus (pH 5.4), whereas the corresponding rab7 mutant (late endosomes) only blocked influenza virus entry.

The progress of individual virus particles through endocytic compartments can be tracked with real-time video microscopy (41–44). Individual fluorescent virus particles can be observed to bind to the cell surface, diffuse along the membrane, get trapped in coated pits or caveolae, enter by endocytosis, move along microtubules, and so on. With the use of specific fluorescent dyes, the acidification of virus particles and the fusion of the viral envelope with cellular membranes can be monitored.

Lipid Raft–Mediated Endocytosis of Viruses

SV40 and some other viruses choose endocytic pathways that bypass clathrin-mediated endocytosis. One of these involves caveolae, flask-shaped indentations of the plasma membrane enriched in cholesterol and sphingolipids, caveolins, and signaling factors (45–48). Although generally immobile, caveolae are known to support the internalization of certain physiological ligands (49–51).

After binding to the GM1 ganglioside, SV40 moves laterally along the plasma membrane until trapped in a caveolae (42, 52) (Fig. 2). Entry proceeds through a caveolar vesicle, the caveosome, and then the smooth ER. Penetration into

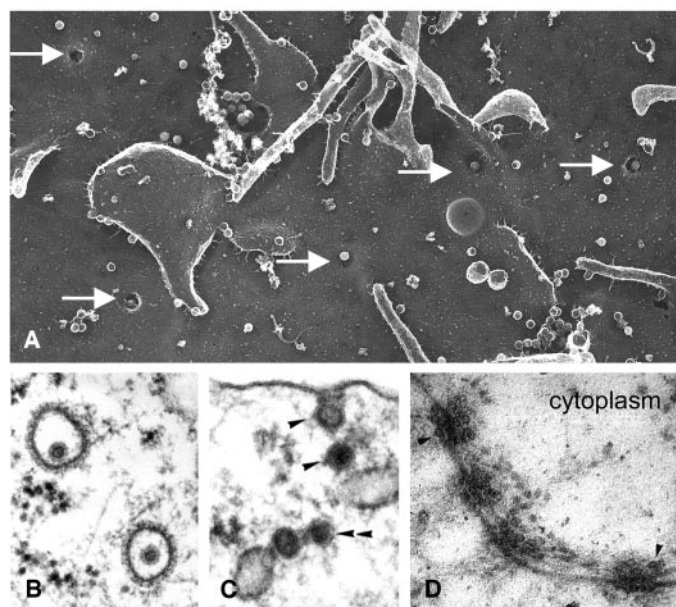


Fig. 1. Electron micrograph of virus entry. (A) Semliki Forest virus, a simple enveloped toga (alpha) virus, binds to the surface of baby hamster kidney (BHK-21) cells in large numbers. Some of the viruses are associated with microvilli, whereas others are localized in indentations that correspond to clathrin-coated pits (arrows). [Courtesy: J. Heuser and A. Helenius] (B) Semliki Forest virus particles in clathrin-coated vesicles. [Courtesy: J. Kartenbeck and A. Helenius] (C) SV40 particles (arrowheads) are internalized by tight-fitting caveolar vesicles and transported to caveosomes that contain multiple viruses. [Courtesy: J. Kartenbeck and A. Helenius] (D) The capsids of human hepatitis B viruses (arrowheads) pass through the NPCs in transit from the cytoplasm to the nucleoplasm. In the experiment illustrated in this micrograph, recombinant capsid-like particles were micro-injected into *Xenopus* oocytes, and their interaction with the nuclear pores visualized in electron micrograph sections. Rows of capsids lined up within the NPCs (arrows). [Courtesy: N. Pante and M. Kann]

the cytosol is thought to occur in the ER, after which the virus enters the nucleus by way of the nuclear pore complexes (NPCs). This pathway has many interesting and unexpected features [for reviews, see (53–56)]. Caveolae are also used by the polyoma virus in some cell types, papilloma viruses, and Echo 1 virus (57–60).

In addition to the caveolae, it is evident that cells have other clathrin-independent pathways of endocytosis (55, 61). These noncaveolar, lipid raft–dependent pathways are still poorly characterized. They may serve as a primary entry route for viruses such as polyoma (59, 62) and as an alternative entry route for SV40 in cells that lack caveolae (63).

The presence of multiple pathways and previously unobserved endocytic organelles challenges established assumptions about the entry of many viruses. Cellular processes can be more complex than anticipated, which is illustrated by the recent observation that influenza virus, which was thought to enter by clathrin-coated pit endocytosis, can infect cells in which clathrin-coated vesicle transport is blocked (64).

Penetration

Penetration of enveloped viruses occurs by membrane fusion catalyzed by fusion proteins in the viral envelope. The machinery involved is rather simple, at least when compared to the apparatus needed for intracellular membrane-fusion events. One reason for simplicity is that viral fusion factors are used only once. Fusion activity is triggered by cues in the form of receptor binding or low pH (as mentioned above). They induce, as a rule, irreversible conformational changes.

Viral fusion factors are currently divided into two main classes. Type I factors consist of homotrimeric spike glycoproteins in which the subunits are joined by long coiled coils. They are membrane proteins that are synthesized as precursor proteins, folded, and assembled into oligomers in the ER. In transit through the secretory pathway, they undergo postsynthetic proteolytic cleavages that render them conformationally metastable and fusion competent (4, 65). Their metastable state allows cooperative conversion into a lower energy conformation. When triggered, the resulting conformation exposes hydrophobic fusion sequences that insert into the target membrane. The released free energy is used to force the membranes closer together in a focal site, resulting in fusion. Influenza HA is the best studied in this class. For more information about this well-studied process, see recent reviews (5, 7, 66, 67).

Type II fusion proteins occur in flaviviruses and in alpha viruses (68, 69). They have internal fusion sequences and are synthesized and assembled as heterodimers with another membrane protein. When exposed to low pH, a change occurs in quaternary structure; the fusion subunits disso-

ciate from their partners and join together as active homotrimers (69–71).

Membrane fusion is an elegant and effective way to deliver viral capsids into the cytosol. No macromolecular assemblies need to pass through a hydrophobic membrane barrier. The underlying principle is the same as in intracel-

lular membrane traffic; the viral envelope is a “transport vesicle,” and the capsid is the cargo.

Because nonenveloped viruses do not have a membrane, they penetrate either by lysing a membrane or by creating a porelike structure in a membrane. Although details remain obscure, it is clear that penetration of

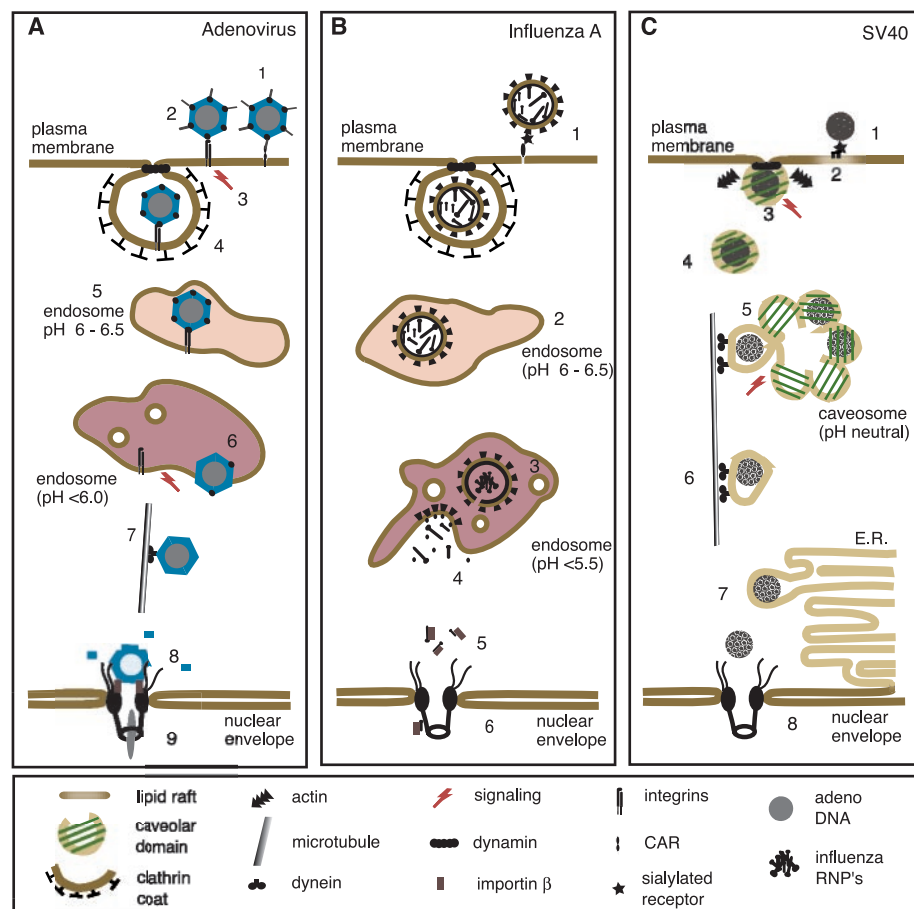


Fig. 2. Entry of adenovirus, influenza virus, and SV40 into their host cells. **(A)** Entry of adenovirus. Adenoviruses are non-enveloped DNA viruses with an icosahedral capsid composed of hexon proteins, penton base complexes, and homotrimeric fibers. The entry process includes the following steps (82–84): (1) The fibers bind to the CAR. (2) Fibers dissociate, and the pentons expose RGD sequences that bind to integrins. (3) Clustering the integrins activates phosphatidylinositol-3-OH kinase, Rac, and cdc42, resulting in cytoskeleton rearrangements. (4) The virus-integrin complex internalizes in clathrin-coated vesicles. (5) The virus is transported to early endosomes. (6) At a pH of 6, the penton base undergoes a conformational change and the virus escapes into the cytosol by endosomal lysis. (7) The virus particles bind dynein and are transported along microtubules to the NPC. (8) Capsids dock at the NPC protein CAN/Nup214, disassemble, and (9) release the viral DNA for transport through the pore. **(B)** Entry of influenza virus A. Influenza A is an enveloped negative-stranded RNA virus. Entry proceeds through endosomes by means of the following steps (37, 85, 108, 109): (1) Viral HA binds to sialic acid-containing glycoproteins or glycolipids. (2) Viruses internalized by clathrin-coated pits are transported by way of the early endosomes to the late endosomes. (3) Low pH activates the M2 protein ion channel in the viral membrane, allowing the internal capsid to be acidified. (4) HA-mediated fusion occurs between the viral envelope and the endosomal membrane triggered by low pH (~5.5). (5) Viral ribonucleoproteins (RNPs) separate from each other, bind importin β , and move through the NPC and (6) into the nucleus. **(C)** Entry of SV40. SV40 is a non-enveloped DNA virus that replicates in the nucleus. Its entry steps are partially known and include the following (53–56): (1) SV40 binds to gangliosides in the plasma membrane, and (2) is included into lipid rafts. (3) After sequestration into caveolae, activation of tyrosine kinases induces local phosphorylation that results in F-actin dissociation, actin accumulation around the caveolae, dynamin 2 recruitment, and activation of caveolar endocytosis. (4) Virus-containing caveolae are internalized and delivered to caveosomes. (5) The virus induces formation of caveolin-free vesicles and (6) is transported by dynein by means of microtubules to the smooth ER. (7) The virus penetrates the cytosol from the ER, and (8) nuclear import occurs through NPCs.

non-enveloped viruses also involves cooperative changes in virus particles triggered by receptor binding or low pH (16, 72, 73). The viruses become more hydrophobic and interact with membranes directly.

In adenoviruses, the penton base becomes lytic at low pH, and the virus is released from ruptured endosomes intact with the rest of endosomal contents (74, 75). A variation of the same theme is used by reoviruses, in which a hydrophobic peptide in capsid protein $\mu 1$ is exposed, making the capsid lytic (76). In the case of picorna viruses, receptor binding to the canyon leads to loss of the VP4 protein and exposure of the myristic acid group at the N terminus of VP1. The virus is thought to sink into the bilayer and form a protein-lined "channel" through which the viral RNA can enter the cytosol (72).

Intracellular Transport

After penetration, the genome of most viruses must be transported either to the nucleus or to specific cytosolic membranes. Diffusion in the crowded and highly structured cytosol is not efficient given the large dimensions of most capsids and the long distances they must travel (77, 78). To move inside the cell, incoming viruses often exploit the cytoskeleton and cellular motor proteins. As recently reviewed (77), there are two main ways to do this; the viruses can allow endocytic vesicles to ferry them as passive luminal cargo, or the penetrated capsid can itself interact with the relevant motors. In the latter case, a capsid protein binds and interacts with the cellular factors. Transport to the nucleus generally involves the minus-end-directed microtubule-dependent motor dynein and its adaptor protein, dynactin.

Actin can also play a role in virus entry. Because it is rich in actin filaments, the cortical cytoskeleton poses a barrier against the inward movement of capsids and viruses that enter directly through the plasma membrane (79). To overcome the barrier, some viruses, such as SV40, activate tyrosine kinase-induced signaling cascades that lead to the local dissociation of filamentous actin (52).

Actin has also been found to promote vesicle budding at the cell surface and to propel virus-containing endocytic vesicles through the cytoplasm (44, 52). The release of baculovirus from endosomes induces polymerization of actin to one end of the baculovirus capsid, which promotes capsid movement toward the nucleus (80). One of the capsid proteins (p78/83) has homology with the mammalian Wilkott-Aldrich syndrome protein (WASP) and is thus likely to interact with Arp2/3, a protein complex involved in actin assembly (81).

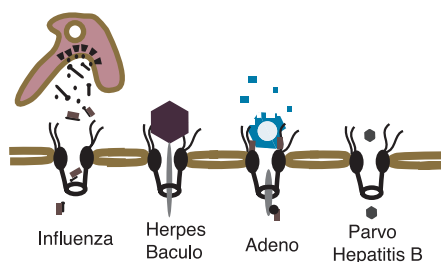


Fig. 3. Import of viruses and capsids into the nucleus. Four different ways are shown by which viruses use the NPCs for import. **(Left)** The genome of influenza virus is contained within eight subgenomic RNAs that are individually packaged into ribonucleoprotein complexes. Once these are released into the cytosol after viral membrane fusion, they interact with importin β and are imported into the nucleus. They are small enough to pass through the NPC. **(Middle left)** The capsids of HSV-1 bind to the cytosolic side of the pore, a vertex in the capsid is opened, and the DNA passes through the pore leaving behind an intact empty capsid. **(Middle right)** Adenoviruses also bind to the pore but then undergo disassembly. The viral particles dock to the NPC protein CAN/Nup214 and disassemble with the help of histone H1. The viral DNA with terminal covalently bound proteins is released and enters the nucleus. **(Right)** Parvoviruses and the capsids of hepatitis B virus are small enough to pass through the pore in intact form. Uncoating occurs in the nucleus.

ceptors, integrins, and gangliosides) and can be activated by virus binding or virus-induced clustering. SV40 and adenovirus family C members base their entry strategy entirely on signaling (Fig. 2). By activating tyrosine kinases in caveolae, SV40 triggers the normally dormant, ligand-inducible caveolar endocytosis pathway. A second signal is induced in the caveosome to induce caveosome-to-ER transport (55).

By clustering its entry receptors, adenovirus 2 activates a variety of protein kinases, phosphatidylinositol-3-OH kinase, and small GTPases (82–84). Major changes occur in the cell surface dynamics, and in microtubule-mediated transport, which promote internalization, penetration, and intracellular traffic of the incoming virus. Human cytomegalovirus, a herpes virus, activates several signaling pathways through the interaction between envelope glycoprotein B and the epidermal growth-factor receptor (85).

Signaling During Virus Entry

In recent years, it has become clear that the information exchange between incoming viruses and the host cell is not limited to cues given to the virus by the cell. For many viruses, it takes the form of a two-way dialogue in which the virus takes advantage of the cell's own signal transduction systems to transmit signals to the cell (82, 83). These signals induce changes that facilitate entry, prepare the cell for invasion, and neutralize host defenses.

The signals are usually generated at the cell surface through the virus binding to receptors that are themselves signaling molecules or modulators (e.g., growth factor receptors, chemokine receptors,

Nuclear Import

The nucleus provides excellent "service" functions for virus replication, ranging from DNA and RNA polymerases to RNA-splicing and -modifying enzymes. However, the nucleus is difficult to enter and exit, and viruses must again rely on cellular mechanisms [for reviews, see (56, 86, 87)].

In interphase cells, the import of virus and viral capsids occurs through the NPCs (Fig. 3). For targeting, viruses use nuclear localization signals and cytosolic import receptors. HIV-1 and adenovirus bind to importin 7, and human papilloma viruses 11 and 45, hepatitis B virus capsids, and influenza virus nucleoproteins are known to bind importins α and β (88–92).

Recent studies show that the upper limit in particle diameter for transport through the NPC is 39 nm (93). The smallest viruses and capsids, as well as helical capsids in extended form, can therefore be imported into the nucleus without disassembly or deformation. Among icosahedral particles, parvoviruses (diameter 18 to 24 nm) and the capsids of hepatitis B virus (diameter 36 nm) are probably imported intact (94). The capsids of hepatitis B virus are uncoated in the basket on the nuclear side of the pore complex (95).

Larger viruses and capsids must either be deformed or disassemble to allow the genome to pass through the NPC. Adenovirus 2 binds to NUP214/CAN, a protein located at the base of the filaments extending into the cytosol from the nuclear pore (94) (Fig. 2). Interaction of the bound virus with histone H1 and importins β and 7 induces disassembly of the virus capsid. The viral DNA thus liberated is imported into the nucleoplasm. The HSV-1 capsids (diameter 120 nm) also bind to the NPCs in an importin β -dependent manner, but the DNA is released through one of the icosahedral vertices of the capsid without further capsid disassembly (96, 97). The empty capsid stays bound to the pore complex for hours after the DNA has been ejected (98).

With the exception of lentiviruses such as HIV-1, retroviruses do not use the NPCs for nuclear entry. Preintegration complexes can only enter the nucleus during mitosis when the nuclear envelope is temporarily absent, limiting their infectivity to dividing cells. The molecular basis for nuclear uptake of lentivirus preintegration complexes is not entirely clear, but there is evidence that three viral proteins are important: the matrix protein, the integrase enzyme, and the small accessory protein vpr. In addition, it is reported that a short triple-stranded overlap in the provirus DNA may be essential at least in some cells. For more detailed discussion of this topic, see (87, 99, 100).

Direct Cell-to-Cell Transfer with and Without Virus Infection

Finally, infection can be transmitted directly from cell to cell. Viruses such as measles, which

express in the plasma membrane envelope proteins that are fusogenic at neutral pH, often induce fusion of infected cells with uninfected neighboring cells. Thus, the viral genes pass directly from cell to cell, and infection occurs without the involvement of viral particles.

In an alternative strategy for cell-to-cell transfer, extracellular vaccinia virus particles are practically pushed into an adjacent cell by localized actin polymerization on the inside of the infected cell (101). The polymerization of actin is triggered by a viral protein that recruits the plasma membrane WASP, Arp2/3, and other cellular factors that promote actin polymerization.

The observation that HIV-1 and other lentiviruses in some cell types bud into endosome-like vacuoles has raised the possibility that virus particles can be released by the infected cell in a polarized fashion by means of regulated secretion (102). HIV-1 particles may, in this case, be directly transmitted from a macrophage to a T cell as part of the normal cell-to-cell interaction. There is also evidence that dendritic cells, without getting infected, may concentrate HIV-1 in regions of cell-to-cell contact and thus promote infection (103, 104).

Perspectives

Viruses continue to pose a serious threat to the life and well-being of humans, animals, and other organisms. In the past, the search for antiviral drugs was focused mainly on replicases and other viral enzymes. That entry and uncoating can serve as a target for antivirals has recently been demonstrated by new inhibitors against influenza neuraminidase and the fusion protein of HIV-1 (105, 106). With our information rapidly reaching the molecular level, it may be possible to develop new approaches to block the entry of viruses (107).

Taking advantage of their capacity to enter cells and express their genes, viruses are used as vectors for the expression of recombinant proteins in cells and for the production of proteins. In gene therapy, viruses are used to deliver genes to cells and tissues. Although there are still many problems with this form of therapy, more information about virus receptors and entry mechanisms will help to make viruses safer and more useful as therapeutic tools.

Analysis of viruses has traditionally provided insight into basic principles of gene expression, molecular biology, and cancer. Today, viruses are still powerful tools in many areas of research, including cell biology, molecular biology, and immunology. Careful analysis of early virus-cell interactions is likely to extend our still incomplete understanding of plasma membrane dynamics, membrane fusion, endocytic pathways, and many other aspects of cell function.

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REVIEW

Bacterial Invasion: The Paradigms of Enteroinvasive Pathogens

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Invasive bacteria actively induce their own uptake by phagocytosis in normally nonphagocytic cells and then either establish a protected niche within which they survive and replicate, or disseminate from cell to cell by means of an actin-based motility process. The mechanisms underlying bacterial entry, phagosome maturation, and dissemination reveal common strategies as well as unique tactics evolved by individual species to establish infection.

To establish and maintain a successful infection, microbial pathogens have evolved a variety of strategies to invade the host, avoid or resist the innate immune response, damage the cells, and multiply in specific and normally sterile regions. Based on their capacity to deal with these critical issues, bacteria can be grouped in different categories. Here we review the so-called invasive bacteria, i.e., bacteria that are able to induce their own phagocytosis into cells that are normally nonphagocytic. We focus on the tactics used by enteroinvasive bacteria to trigger their uptake by epithelial cells and discuss their intracellular life-styles. The mechanisms of entry and life-styles of other intracellular pathogens have been reviewed elsewhere (1–4).

During phagocytosis by phagocytes, bacteria play a passive role. In contrast, during bacterial-induced phagocytosis, the bacterium is the key and active player in the complex interplay between the invading microbe and the host cell (5). Another important component is the cytoskeleton, whose plasticity is critical and optimally exploited. After internalization, some bacteria remain in a vacuole, in which they replicate. They prevent the normal maturation and trafficking of the phagosome and impair its normal bacteriolytic activities. Other bacteria escape from the vacuole and replicate in the cytosol. In some cases, they also move and disseminate by means of an actin-based motility process.

How the cell senses the bacterial intruders and adjusts its transcription and translation programs to its new life with a parasite is an important issue. Apoptosis and anti-apoptosis, as well as cell cycle- and inflammation-related signaling pathways, are reprogrammed after infection to help the cell to survive the stress induced by the infection.

The success of an infection depends on the messages that the two players—the bacterium and the cell—send to each other. At each step of the infectious process, the bacterium exploits the host cell machinery to its own profit.

Entry Mechanisms

To enter nonphagocytic cells such as intestinal epithelial cells, some microbial pathogens express a surface protein able to bind eukaryotic surface receptors often involved in cell-matrix or cell-cell adherence. Expression of this protein leads to the formation of a vacuole that engulfs the bacterium through a “zippering” process in which relatively modest cytoskeletal rearrangements and membrane extensions occur in response to engagement of the receptor. The initial interactions between the bacterial protein and its receptor trigger a cascade of signals, including protein phosphorylations and/or recruitment of adaptors and effectors, and activation of cytoskeleton components that culminate in phagocytic cup closure and bacterial internalization. Other pathogens have devised mechanisms to bind a protein that can itself act as a bridge between the bacterium and a transmembrane receptor, which then mediates the entry process. Finally, pathogens can also bypass the first step of adhesion and interact directly with the cellular machinery that regulates the actin cytoskeleton dynamics by injecting effectors through a dedicated secretory system. The effector molecules cause massive cytoskeletal changes that trigger the formation

of a macropinocytic pocket, loosely bound to the bacterial body.

The Zipper Mechanism of Entry

Yersinia pseudotuberculosis and *Listeria monocytogenes* both harness transmembrane cell-adhesion proteins as receptors for entry into mammalian cells (Figs. 1A and 2A). Entry can be divided into three successive steps: (i) Contact and adherence. This step is independent of the actin cytoskeleton and involves only the bacterial ligand and its receptor. It leads to receptor clustering. (ii) Phagocytic cup formation. This step is triggered by the transient signals occurring after formation of the first ligand-receptor complexes and propagating around the invading microbe. These signals induce actin polymerization and membrane extension. (iii) Phagocytic cup closure and retraction, and actin depolymerization.

The *Yersinia* outer-membrane protein invasin binds to integrin receptors that have the β_1 chain and are normally implicated in adherence of cells to the extracellular matrix (6). Invasin does not possess the RGD motif present in fibronectin, but both proteins interact with integrins by a structurally similar domain. Invasin has a higher affinity for integrins and can oligomerize, inducing integrin clustering and efficient downstream signaling. The cytoplasmic tail of the β_1 chain, which normally interacts with the cytoskeleton in focal complexes of adhesion plaques, is critical for entry, but surprisingly, alterations of this domain that impair interaction with the cytoskeleton increase internalization. Thus, a lower affinity of the integrin for the cytoskeleton could allow higher mobility of the receptors in the membrane.

Activation of integrins leads to tyrosine-phosphorylation events required for entry. The tyrosine kinase FAK (focal adhesion kinase) is the most attractive candidate for transmitting a signal from clustered integrins to the cytoskeleton, because the β_1 -chain cytoplasmic domain binds to FAK, and dominant-inhibitory mutations in FAK strongly impair invasin-mediated uptake (7). Src, phosphoinositide 3-kinase (PI 3-kinase), and

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