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Virus Entry: Looking Back and Moving Forward

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Abstract

Research over a period of more than half a century has provided a reasonably accurate picture of mechanisms involved in animal virus entry into their host cells. Successive steps in entry include binding to receptors, endocytosis, passage through one or more membranes, targeting to specific sites within the cell, and uncoating of the genome. For some viruses, the molecular interactions are known in great detail. However, as more viruses are analyzed, and as the focus shifts from tissue culture to in vivo experiments, it is evident that viruses display considerable redundancy and flexibility in receptor usage, endocytic mechanism, location of penetration, and uncoating mechanism. For many viruses, the picture is still elusive because the interactions that they engage in rely on sophisticated adaptation to complex cellular functions and defense mechanisms.

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Looking back

When my collaborators and I began to investigate mechanisms of animal virus entry in 1976, several key concepts were already known [1]. Some of them had been established with T-even bacteriophages, which at the time had been studied in much greater detail than animal viruses. The phages were known to have proteins that bound to cell surface "receptors" on specific host bacteria. Interaction with the receptors triggered conformational changes in the virus particles. The result was perforation of the bacterial cell wall and ejection of the viral DNA genome and some proteins into the cytosol.

Being much simpler in structure and composition, animal viruses were not likely to use a bacteriophage-like ejection mechanism. However, influenza and paramyxoviruses were known to use sialic acid residues as receptors, and have sialidase activity for receptor destruction [2]. It was generally assumed that other animal viruses used a variety of receptors explaining the observed differences in cell tropism and host range. Identification of additional receptors took place later: The HIV virus receptor CD4 on T cells and the Epstein–Barr virus receptor CR2 on B cells were identified in 1984 [3,4], the poliovirus receptor CD155 in 1989 [5], and the coxsackie adenovirus virus receptor in 1997 [6].

The major controversy in the emerging field of animal virus entry at the time was whether viruses—once bound to their receptors—penetrated directly through the plasma membrane or only after endocytosis (called "viropexis" at the time) [7]. Envelope glycoproteins in members of the paramyxovirus family were known to possess cell–cell fusion activity [8]. This suggested that penetration of enveloped viruses relied on fusion of the viral membrane with the plasma membrane. However, other mechanisms could not be excluded [9]. The endocytic pathway was dismissed by many as a mechanism that cells employed to defend themselves by destruction of viruses in lysosomes.

The available data were confusing and contradictory. To take an example, vesicular stomatitis virus was shown by electron microscopy (EM) to undergo endocytosis in L cells through what we now recognize as clathrin-coated pits and vesicles [10,11]. Equally convincing-looking electron micrographs showed direct fusion of the viral envelope with the L cell plasma membrane [12].

It was known, moreover, that some viruses underwent a stepwise uncoating process during entry culminating in the release of the genome. This insight was based primarily on picorna virus work [13]. After association with cells, these viruses could be shown to undergo what was called "eclipse," that is, a series of conformational changes before the viral RNA was released. Incoming adenovirus capsids were known to associate with microtubules and with nuclear pore complexes followed by delivery of the genome into the nucleus [14].

However, as commented by Sam Dales, one of the pioneers, all these facts were “established with only a modest degree of credibility” [15]. The main problem was that most of the studies relied on EM. With EM, it was not possible to distinguish between productive and non-productive entry pathways and infective and non-infective particles. For many viruses, the high particle to plaque-forming unit (a measure of virus infectivity) ratio makes analysis of entry difficult still today. In the early 1980s, it was in addition hard to obtain funding as major agencies had written off virus entry as “unsolvable.”

Only by combining morphological and biochemical methods with perturbants and inhibitors that affected the pathway to productive infection did it become possible to make progress. In our own early experiments, we used weak bases such as ammonium chloride and chloroquine as perturbants [16]. Although their mechanism of action remained unknown, these agents were found to inhibit entry of several virus families [17]. Only later was it established that they raise the pH of acidic organelles in the cell [18]. The virus families affected turned out to be those in which penetration was triggered by low pH.

In combination with cell entry and in vitro fusion experiments, the inhibitors allowed us to demonstrate that endocytosis is essential for entry of several enveloped virus families and that fusion activity of the envelope proteins is triggered by low pH [16,19]. It became clear that to understand virus entry, it was critical to learn about cell biology and membrane biology. Also, it was evident that viruses could serve as useful tools and model systems to study cellular phenomena such as endocytosis, membrane trafficking, and membrane fusion.

In the main stream of virus research

Today, entry studies constitute a major subfield in virology as illustrated by the collection of reviews and primary publications in this volume, and by more than 1500 reviews and 20,000 publications in the literature. The entry of hundreds of viruses has been analyzed in tissue culture cells and increasingly in vivo. The methods used range from detailed structural biology of receptor/virus interactions to live cell imaging of incoming viruses, loss-of-function screens, and mathematical modeling. The level of detailed information available is impressive.

My purpose in this review is to describe the general framework of current concepts in the field. Since there are numerous reviews and book chapters on this topic as well as on individual viruses and virus families, I will focus on a few novel issues.

Regarding the old controversy mentioned above, we now know that the majority of virus species use...
endocytosis. Several different endocytic mechanisms can be used (Table 1). However, some enveloped viruses can penetrate directly through the plasma membrane by membrane fusion. Being pH independent, such fusion reactions are triggered by interactions with one or more cell surface receptors. HIV-1, a lentivirus, needs two cell surface receptors that induce successive changes in the conformation of the fusion-active glycoprotein. In the case of paramyxoviruses and herpes viruses, fusion is usually triggered by interactions between receptor-binding viral spike glycoproteins and distinct metastable viral fusion proteins [20]. For the fusion activation mechanism, different models are being discussed [21].

Since direct fusion at the plasma membrane and endocytosis and intracellular fusion occur in parallel, it is not always easy to determine how much each of them contributes to infection [22]. For example, HIV-1 is able to fuse at the cell surface and intracellularly, but there is evidence that fusion at the plasma membrane does not progress beyond the lipid-mixing stage [23]. The same is true for influenza A virus when fusion at the plasma membrane is induced by low pH [24]. Here, capsid release and uncoating fail to occur. Thus, conclusive demonstration that fusion at the plasma membrane actually leads to infection is still pending for many viruses.

### Entry in several steps

For viruses that enter by endocytosis, the pathways are complex. The viruses depend on the dynamics of the plasma membrane, membrane trafficking, signaling, endosome maturation, and a variety of other cell functions [25]. The overall program can usually be broken down into consecutive steps: (1) attachment to the cell surface; (2) lateral movement along the plasma membrane and receptor clustering; (3) activation of cellular signaling pathways; (4) endocytosis and transport to secondary organelles; (5) penetration by membrane fusion, lysis, or channel/pore formation; (6) intracellular transport into the nucleus or location within the cytoplasm; and (7) partial or complete uncoating of the virus particles or capsids in the cytosol, at the nuclear pore, or in the nucleoplasm.

It is important to realize that the steps listed above leave a lot of room for variability. The receptors on the cell surface are generally different between viruses, the signaling pathways are distinct, several different endocytic machineries can be activated, and there are many mechanisms of penetration. Uncoating has been studied in detail for just a few viruses, but what has been learned is that the processes are variable, complicated, and full of surprises [26–28].

### Receptors and membrane domains

Among the most important virus–host interactions are those between the incoming virus and receptors and co-receptors in the plasma membrane. Some of the contacts provide attachment only, while others promote signaling, induce plasma membrane ruffling, activate endocytosis, and trigger changes in the viral particle [28]. For many viruses, these early events are defining features for species and cell tropism in vivo and in tissue culture [29]. While the presence of attachment factors and receptors alone does not guarantee infection, it is clear that cells that do not support binding of a virus cannot be infected. That infection is limited to specific tissues and cell types and has many consequences. For example, it determines the pathogenesis of disease, it limits the damage caused by the infection on the host, and it defines mechanisms of transmission. In addition, the receptors hold the key to many downstream events in entry such as signaling, endocytosis, penetration, and uncoating.

Without discussing the role of receptors during virus entry—a huge topic by itself—in further depth, there is one emerging aspect that I would like mention. It is related to the fact that the plasma membrane contains a dynamic mosaic of domains and microclusters of different size and composition [30,31]. Being multivalent, viruses are likely to have a higher probability of binding to microdomains that contain pre-clustered receptors, and to induce

### Table 1. Main mechanisms of animal virus endocytosis

| Clathrin-mediated endocytosis | Macropinocytosis | Micropinocytosis | Caveolar endocytosis |
|-----------------------------|-----------------|-----------------|---------------------|
| Small- and medium-sized viruses (alpha, flavi, rhado, etc.) | Used by many larger viruses (vaccinia, Ebola, human cytomegalovirus, etc.) | Small- and medium-sized viruses (papilloma, influenza, etc.) | Small, non-enveloped viruses (Simian virus 40, polyoma, etc.) |
| Clathrin-coat assembly is often induced locally by virus particle | Activation of receptor tyrosine kinases or integrins | Viruses trigger formation of small, uncoated vesicles | Viruses activate caveolar vesicle formation |
| Some viruses associate with pre-existing coated pits | Plasma membrane ruffling or blebbing is induced | Dependent on actin, PAK1, Na+/H+ exchanger, etc. | Uptake is dependent on cholesterol in plasma membrane |
| Uptake usually within minutes of virus binding | Formation of large, fluid-filled vacuoles | Independent of Rho and Rac1 | Dynamin-dependent |
| Virus cargo delivered to early endosomes | Dependent on actin, cdc42, Rac 1, and Na+/H+ exchanger | Virus cargo delivered to early endosomes | Virus cargo delivered to early endosomes |
| Depends on dynamin, clathrin-adaptors, and clathrin | | | |
further clustering or remodeling of such domains. The outcome may result in formation of “platforms” that can trigger virus modification, endocytosis, signaling, and other events [32].

The issue of microdomains is particularly relevant for a wide range of viruses including HIV-1, human cytomegalovirus, human papilloma virus, hepatitis C, influenza, and corona viruses, that depend on tetraspanins such as CD9, CD63, CD81, and CD151 for efficient infection [33]. In the plasma membrane, tetraspanins are known to organize locally into dynamic, ordered clusters and microdomains (tetraspanin-enriched microdomains) that contain, in addition to tetraspanins, selections of surface proteins [34,35]. Tetraspanin-enriched microdomains play a role in cell adhesion, migration, fusion, signaling, vesicle traffic, and other processes. Also in virus infection, they seem to have multiple functions including receptor presentation, exposure of viruses to proteolytic activators, signaling, clathrin-independent endocytosis, and post-endocytic events such as endosome maturation [33]. It is not clear to what extent viruses actually interact with tetraspanins directly.

Recent work on Middle East respiratory syndrome virus illustrates some of the consequences of tetraspanins in infection. In addition to its \textit{bona fide} receptor (DPP2), this corona virus requires exposure to protease; either a TTSP family member on the plasma membrane or cathepsins in endolysosomes. These cleave the viral fusion protein and activate membrane fusion. Studies with mouse lung-adapted Middle East respiratory syndrome virus in tissue culture and \textit{in vivo} show that infection efficiency is elevated by tetraspanin CD9 because it links the DPP2 and TMPRSS2 (a TTSP protease) into a ternary complex [36]. That these entry factors are associated with each other and concentrated in microdomains ensures rapid processing of incoming virus, efficient infection, and higher virulence. Clinical isolates of the related human coronavirus-229E also require CD9. Unlike tissue culture adapted strains, they prefer activation by TMPRSS2 on the cell surface over activation by cathepsin L in late endocytic compartments [37]. A likely explanation is that by elevating the concentration of receptor and TMPRSS2 locally, CD9 promotes early activation, which in turn allows entry without passage into late endocytic compartments. The virus avoids inactivation, exposure to interferon induced factors, and detection by Toll-like receptors that can activate cellular innate immunity.

**Endocytosis and endosomes**

It was initially thought that viruses serve as passive cargo for ongoing cellular endocytic processes. It is now apparent that a majority of them trigger internalization by activating endocytic processes such as macro- and micropinocytosis or by inducing clathrin coat formation [22,38,39]. They do this by activating signaling pathways through direct or indirect contacts with cell surface molecules and structures.

In the case of macropinocytosis, activation involves receptor tyrosine kinases, integrins, and other signaling receptors via exposed phosphatidyl serine (PS) in the viral envelope membrane. The PS is recognized by PS-binding proteins such as members of the TIM/TAM family [39]. Transmembrane signals can be also triggered by receptor clustering and perhaps by induction of membrane curvature. Comprehensive loss-of-function screens (e.g., siRNA, CRISPR-Cas9, etc.) and other studies indicate that hundreds of cellular proteins are involved in the complex signaling, membrane deformation, and vesicle scission events [40,41].

When endocytosis first emerged as a mechanism of virus entry, little was known about the pathway from the primary endocytic vesicles to the final destination, the lysosome. To illustrate the prevailing view of receptor-mediated endocytosis, I have chosen a cartoon from 1980 drawn by Pierre De Meyts, who worked on hormone uptake (Fig. 1) [42]. To those of us who followed viruses after endocytosis, it was clear that coated vesicles did not deliver the virus particles and other cargo directly to lysosomes but rather to uncoated, large vacuoles largely devoid of luminal material. We started calling these \textit{endosomes}, a term still used today for these important organelles [43]. We could later demonstrate that being acidic they were sites of virus penetration [44].

**Penetration**

The transfer of a virus or a capsid through a membrane into the cytosol constitutes a critical step in the entry program. When the particle has reached the right location within the cell, the viral particle or components of the virus execute the penetration process often with assistance from cellular factors [25]. The location can be the plasma membrane, the organelles of the endocytic network (early endosome, late endosome, maturing endosome, recycling endosome, macropinosome, and endolysosome), or an organelle connected through membrane traffic with the endocytic network such as \textit{trans}-Golgi network and endoplasmic reticulum.

Most commonly, the cues that trigger penetration include low pH in endocytic vacuoles, specific molecular interactions with receptors, proteolytic cleavages, and interaction with molecular chaperones [28]. The cues induce changes in the viruses or in metastable viral proteins activating the penetration modus. Enveloped viruses penetrate by membrane fusion, non-enveloped virus by membrane lysis or by the formation of transmembrane pores of different sizes.
Viral membrane fusion proteins were the first fusogenic proteins identified and analyzed [45]. Detailed studies over many years have provided evidence for a general mechanism of action, in which the fusion proteins first bind to the target membrane and bridge the gap between the two membranes. By undergoing further conformational changes, they force the two bilayer surfaces locally together resulting in hemifusion followed by full fusion and stalk-pore formation [46,47].

When cellular fusion proteins were later identified and analyzed in presynaptic vesicle fusion and other intracellular fusion events, it was found that they work by a similar mechanism [48]. It is now clear that some cellular proteins involved in cell–cell fusion events, in fact, represent structural homologs of class I and II viral fusogens [49]. Syncytins that form syncytiotrophoblasts during placenta development are derived from retroviral class I viral proteins [50,51]. The FF proteins involved in cell–cell fusion in nematodes are structural homologs of class II viral proteins. Most recently, HAP2 in unicellular eukaryotes and flowering plants has been found to fuse gametes. It has a structure similar to class II viral fusion proteins [52,53].

The role of endosome maturation

The endosomal network comprises organelles with multiple functions, cellular locations, and properties. Due to the logistics of the network, organelles do not represent fixed entities: they undergo a variety of fusion/fission events and a complex maturation process that alters their characteristics dramatically [54,55]. In addition to gradual drop in intraluminal pH of endosomes, the process involves intraluminal vesicle formation, transport to the perinuclear region, Rab-switching, phosphatidyl inositolphosphate conversion, acquisition of lysosomal proteins and hydrolases and many other changes before they finally fuse with lysosomes.

An incoming virus particle is therefore exposed to a continuously changing environment, and it has to respond to it properly. When the trigger for membrane fusion and penetration involves low pH, the threshold pH is the main factor defining the location and timing of the penetration event [56–58]. Rhabdo- and alphavirus undergo acid-activated fusion in early endosomes where the pH is about 6.2. When the threshold pH is below 6.0 as it is for influenza A virus, delivery to late compartments is required. For such late penetrating viruses timing becomes critical because the viruses risk inactivation by proteases prior to penetration [59].

The pH in endosomes is regulated in complex ways by cellular factors [60]. Interestingly, new studies suggest that some incoming viruses can take advantage of cellular factors to adjust their pH threshold so that fusion can occur earlier [61]. For Lassa virus, this occurs when the virus interacts with an intracellular receptor, LAMP1, a glycoprotein in the endosome/macropinosome membrane [62]. Other viruses have been shown to depend on UVRAG, a cellular protein that controls SNARE complex assembly in homotypic late endosome fusion and delays lysosomal delivery of cargo and possibly exposure to immune recognition [63].

Late penetrating viruses are generally dependent on proper maturation of endosomes and macropinosomes. This means that perturbations that inhibit the process of endosome conversion to late endosome...
(or multivesicular body) block infection. Treatments that affect any component in endosome/macropinosome maturation program can cause entry inhibition because events during maturation are tightly coordinated and interdependent. In our work with influenza A virus, we observed that interference with endosome movement along microtubules or with intraluminal vesicle formation by the ESCRT complexes prevents proper penetration and capsid uncoating [64–66].

### Variation and redundancy

In many recent studies, it is shown that mechanisms and virus cell interactions during entry are more flexible and variable than anticipated from results obtained using tissue culture-adapted virus strains and standard tissue culture cell lines. It turns out that many viruses can use alternative receptors and entry mechanisms depending on cell type and virus strain. For the same virus, entry into highly polarized cell types such as neurons and endothelial cells is, for example, often different from entry into non-polarized cells like fibroblast and T cells. During evolution, viruses have apparently adjusted and fine-tuned their properties to match the life style, physiology, anatomy, and biology of host organisms and host cells. Flexibility, plasticity, and the existence of parallel and alternative pathways are true manifestations of viral life style and a serious challenge in future studies.

Before approaching the issue of redundancy in more detail, it may be useful to consider some examples. From a large number of relevant reports in the literature, I have highlighted some in Table 2. They illustrate various aspects of the phenomenon. Thus, instead of a single fixed entry program, many viruses can utilize alternative receptors and entry pathways [69,78,80]. The mechanisms can occur in parallel in the same cells, or they may operate in different cell types, host species, and under different conditions. Some viruses carry separate surface proteins that allow them to bind to different receptors [69]. Also, like gD in herpes simplex virus, a single surface protein may bind to multiple receptors [81].

Some viruses that can fuse directly at the plasma membrane may in addition employ different forms of endocytosis. Epstein–Barr virus is a good example; a receptor on B cells mediates entry by direct fusion, while another one in epithelial cells leads to micropinocytosis and acid-independent intracellular fusion [67]. Generally, influenza A virus internalization in tissue culture cells occurs by parallel clathrin-mediated endocytosis and micropinocytosis [70]. Sometimes, the activation of penetration by alternative cues, such as low pH or proteolytic cleavage, depend on cell line or virus strain [37]. The pathway by which the virus negotiates the endocytic network in the cell may involve alternative routes and penetration compartments as shown by the rhinovirus example in Table 2 [79]. Viruses continue to evolve by adapting to changing conditions and host cells. This happens for example during adaptation to tissue culture or vaccine production [82,83]. For RNA viruses, the mechanism usually involves accumulation of adaptive mutations and enrichment of mutant quasi-species with better fitness [84]. Adaptation may not only manifest itself by increased binding to attachment factors such as heparin sulfate proteoglycans, but also by receptor switching and changes in host range [85]. As viruses acquire properties that make them more efficient in tissue culture, they usually lose infectivity in primary target cells and pathogenicity.

#### Ubiquitous attachment factors

Many viruses make use of attachment factors and receptors that are widely or ubiquitously expressed [86]. By possessing sialic acid binding glycoproteins, paramyxov- and myxviruses provide extreme examples of this. Since sialic acid is present on practically all

| Table 2. Examples of viruses with alternative entry mechanisms |
|--------------------------------------------------------------|
| **Herpes viruses** | Many herpes viruses can bind to multiple receptors via accessory viral proteins or proteins that can bind to multiple receptors [67,68]. Depending on cell type and virus isolate, herpes simplex virus-1 can undergo fusion at the plasma membrane or in macroinosomes. The latter is either pH-independent or acid-activated [69]. Epstein–Barr virus has distinct envelope proteins that define binding to different receptors on B cells and epithelial cells, and distinct entry pathways. |
| **Influenza A viruses** | They use alternative endocytic mechanisms: clathrin-mediated endocytosis; micropinocytosis, and macropinocytosis [70–72]. In addition to sialic acid, the virus needs unidentified co-receptors [73]. The dependence on actin dynamics differs between polarized and non-polarized host cells [74]. |
| **African swine fever virus** | This large DNA virus enters macrophages by macropinocytosis and clathrin-mediated endocytosis [75]. This bunyavirus enters endosomes in dendritic cells using DC-SIGN, a mannose-specific lectin [76]. Uptake is clathrin-mediated. In cell types lacking DC-SIGN, the receptors are not known, but the endocytic mechanism is micropinocytic [77]. |
| **Uukuniemi virus** | Different isoforms of the same receptor support penetration of avian sarcoma and leukemia viruses from distinct endosomes (early endosomes versus maturing endosomes) [78]. |
| **Avian retro viruses** | Serotypes that use ICAM-1 as receptor enter via clathrin-mediated endocytosis and micropinocytosis. Among these, members of the A serotype are routed for acid-activated uncoating in recycling endosomes and members of the B serotype in maturing endosomes [79]. |
| **Rhinoviruses** | EBV has distinct envelope proteins that define binding to different receptors on B cells and epithelial cells, and distinct entry pathways. |
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cell types and hundreds of cell surface glycoproteins and lipids, these viruses can bind almost everywhere. Cell and tissue tropism is, however, often still limited due to requirements for specific glycosidic linkages and modifications of the sialic acids as well as to cell factors such as proteases required for activation of fusion proteins and to host immune responses [87–89]. For influenza virus, there is evidence that attachment to sialic acid alone is not sufficient for infection; one or more specific co-receptors are required [73]. Through these supplementary interactions, the virus may activate downstream receptor tyrosine kinases or other signaling- and endocytosis-activating receptors [90].

Glycosaminoglycans (GAGs) constitute widely distributed glycoconjugates that serve as attachment factors for many different enveloped and non-enveloped viruses in cell culture and in tissues [91]. Although GAGs are generally not absolutely required for infection, they play a significant role in increasing efficiency and influencing cell specificity. In many cases, interaction with these highly negatively charged and heterogeneous surface glycoconjugates is promoted by clusters of positive charges in viral surface proteins. Binding to GAGs is usually followed by association with cognate receptors and co-receptors for productive entry [69]. Increased binding to GAGs often occurs during adaptation of RNA viruses to tissue culture cells, and it often involves loss of pathogenicity [83,92].

Viral glycans interact with cellular receptors

Some cell types carry lectin molecules that capture viruses that have specific glycans. The awkwardly named “dendritic cell specific intercellular adhesion molecule-3 (ICAM-3) grabbing nonintegrin” (DC-SIGN) is one of these lectins [93]. It binds and internalizes a broad range of viruses that carry high mannose N-linked glycans in their envelope glycoproteins. Viruses introduced into the skin through insect bites and other mechanisms take advantage of DC-SIGN in dendritic cells to promote dissemination from peripheral tissues to lymphoid organs [94]. We have observed DC-SIGN clustering by surface-bound bunya viruses in real time followed by internalization via clathrin-mediated endocytosis [76]. In this case, the lectin not only serves as an attachment factor but as an authentic receptor.

Viral heterogeneity

Different physical forms of virus particles such as filamentous versus spherical may in some cases lead to different entry mechanisms. Influenza viruses occur in two forms: spherical and filamentous. While the spherical particles mainly enter via clathrin-mediated endocytosis and micropinocytosis, the larger filamentous forms use macropinocytosis [95,96]. The particles are presumably too large to use the other two mechanisms. A consideration seldom addressed in virus entry studies is the possibility that virus aggregates may enter cells by mechanisms other than single particles [97,98]. Aggregates may exist in the inoculum or they may form on the cell surface during high multiplicity infection.

Summary

It has been possible to uncover many of the basic pathways and mechanisms that viruses use to enter cells. Some are now understood in remarkable detail at a cellular and even atomic level. Progress has been possible through the use of multidisciplinary approaches involving methods and concepts from epidemiology, medicine, cell biology, biochemistry, structural biology, systems biology, and so on.

However, the deceptive simplicity in structure and composition of viral particles and the limited size of viral genomes conceal a remarkable, built-in complexity that allows viruses to exploit and manipulate host cells and organisms in many sophisticated ways. During coevolution with their hosts, they have developed a modus operandi that is based on profound adaptation to the host(s). During cell entry, they profit from deep insights into the broad spectrum of signals and conduits by which cells in multicellular organisms interact with each other and the outside world. Receptors, signaling, endocytosis, and intracellular trafficking are all part of a refined machinery that viruses take advantage of. Viruses make use of their “insider information” not only for entry and replication but also to avoid cellular defenses, to support different routes of transmission from organism to organism and between tissues in the body, to provide back-up systems, and to expand and adjust tropism. That virus/host cell interactions during entry are often redundant and adaptable is a challenge for our efforts to develop antiviral strategies. However, it is an inherent part of the life-style of many viruses.

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