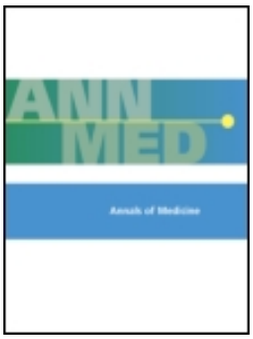

Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: informahealthcare.com/journals/iann20

# Viral entry, lipid rafts and caveosomes

Vilja M. Pietiäinen, Varpu Marjomäki, Jyrki Heino & Timo Hyypiä

**To cite this article:** Vilja M. Pietiäinen, Varpu Marjomäki, Jyrki Heino & Timo Hyypiä (2005) Viral entry, lipid rafts and caveosomes, Annals of Medicine, 37:6, 394-403, DOI: 10.1080/07853890510011976

**To link to this article:** https://doi.org/10.1080/07853890510011976

Published online: 08 Jul 2009.

*Annals of Medicine*. 2005; 37: 394–403

Taylor & Francis
Taylor & Francis Group

**REVIEW ARTICLE**

# Viral entry, lipid rafts and caveosomes

VILJA M. PIETIÄINEN[1], VARPU MARJOMÄKI[2], JYRKI HEINO[3] & TIMO HYYPIÄ[1,4]

[1]*Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland,* [2]*Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland,* [3]*Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland, and* [4]*Department of Virology, University of Turku, Turku, Finland*

**Abstract**
Lipid rafts and caveolae are detergent-insoluble plasma membrane microdomains, involved in cellular endocytic processes and signalling. Several viruses, including a human pathogen, echovirus 1, and an extensively studied simian virus 40 utilize these domains for internalization into the host cells. Interaction of viruses with receptors on the cell surface triggers specific conformational changes of the virus particle and can give rise to signalling events, which determine the mechanisms of virus entry. After internalization via cell surface lipid rafts or caveolae, virus-containing vesicles can fuse with caveosomes, pre-existing cytoplasmic organelles, or dock on other intracellular organelles. These pathways may deliver viruses further to different cellular destinations, where the viral replication cycle then takes place. The information concerning the viral entry processes is important for understanding the details of the infections, for finding new targets for antiviral therapy and for elucidating the cellular internalization pathways in general.

**Key words:** *Caveolae, caveosomes, echovirus 1, lipid raft, simian virus 40, viral endocytosis*

## Early events in virus infection

Viruses consist of a protein capsid surrounding the nucleic acid (DNA or RNA) genome and in some viruses this structure is further covered by a lipid envelope. To initiate infection in the host, the virus must be able to deliver its genetic material into the target cells. This requires specific recognition of cell surface molecules and subsequent entry process. During evolution, viruses have adapted to utilize receptors with different physiological functions for the initiation of their life cycle (Table I) (reviewed in (1)). Specific sites in the virus particle are responsible for these interactions that can include complex recognition events followed by conformational changes in the virus particle and the receptors.

The expression of cell surface molecules varies in different tissues due to the distinct functions of the cells. Specific virus-cell recognition events are essential in viral tissue tropism and pathogenesis of the infection. In some cases the clinical outcome of the infection can be largely explained by the receptor specificity. In Epstein-Barr virus infection, B-cells expressing the complement receptors recognized by the virus (2) are the main target, and in rabies, the virus interacts with the acetylcholine receptor during migration from the exposed peripheral area to the central nervous system (3). However, the pathogenic process is usually complex and in addition to receptor recognition, intracellular interactions between cellular and viral macromolecules play an important role.

Viruses may either use several receptor molecules during the early events (attachment, entry and uncoating) of infection, or, in some cases, only one molecule may be sufficient to bring about all these steps. For instance, human immunodeficiency virus (HIV) is bound to the CD4 molecule on leukocytes but this interaction does not directly lead to the initiation of the infection. Instead, it gives rise to conformational changes in the virus structure allowing further interaction with chemokine receptors and this cascade eventually facilitates the entry of the virus core into the host cells. The same or highly similar cell surface molecules are recognized by different viruses (Table I). Some cell surface

Correspondence: Dr, Timo Hyypiä, Department of Virology, University of Turku, Kiinamyllynkatu 13, FIN-20520 Turku, FINLAND.
Fax: +358-2-251 3303. E-mail: timo.hyypia@utu.fi

ISSN 0785-3890 print/ISSN 1365-2060 online 
DOI: 10.1080/07853890510011976

Table 1. Examples of molecules known to act as virus receptors.

| Receptor | Virus |
|---|---|
| ***Immunoglobulin superfamily*** | |
| CD4 | HIV-1 |
| | Human herpesvirus 7 |
| ICAM-1 | Rhinoviruses (major group) |
| Coxsackievirus-adenovirus receptor | Coxsackie B viruses |
| | Adenoviruses |
| Poliovirus receptor (CD155) | Polioviruses |
| Neural cell adhesion molecule (CD56) | Rabies virus |
| MHC class I | Simian virus 40 |
| Nectin 1 and 2 | Herpes simplex viruses |
| ***Integrins*** | |
| $\alpha$V integrins | Adenoviruses |
| | Coxsackievirus A9 |
| | Human parechovirus 1 |
| | Foot-and-mouth disease viruses |
| | Hantavcrases |
| $\alpha 2\beta 1$ | Echovirus 1 |
| $\alpha 3\beta 1$ | Human herpesvirus 8 (KSHV) |
| ***Other protein receptors*** | |
| Chemokine receptors | HIV-1 |
| Complement receptor CR2 (CD21) | Epstein-Barr virus |
| Decay accelerating factor (CD55) | Echoviruses |
| | Coxsackievirus A21 |
| | Coxsackie B viruses |
| Low density lipoprotein receptor | Rhinoviruses (minor group) |
| Acetylcholine receptor | Rabies virus |
| ***Other molecules*** | |
| Sialic acid | Influenza viruses |
| Heparan sulphate | Herpes simplex viruses |
| Ganglioside GM1 | Simian virus 40 |

molecules (e.g. poliovirus receptor, coxsackievirus-adenovirus receptor) have been primarily identified due to their ability to bind viruses and further studies have elucidated their cellular functions.

After recognition of the cell surface receptor, viruses usually enter the cells utilizing processes which are used for internalization of extracellular material necessary during the physiological life cycle of the cells (reviewed in (4)). The most thoroughly studied mechanism of viral entry is the clathrin-mediated endocytosis pathway (Figure 1). This route is utilized, for instance, by influenza viruses and adenoviruses. After interaction between the virus and the receptor, the virus particles move into clathrin-coated pits and the vesicles are internalized into the cytoplasm. They can fuse with the early endosomes, and the ligand can be further delivered to the late endosomes or to other intracellular organelles. Subsequently, the viral genome needs to be released from the membranous vesicle. This procedure varies between viruses and may take advantage of viral fusion peptides or virus-induced rupture of the vesicle. Some viruses (e.g. HIV and measles virus) are able to enter the cells by direct fusion of the viral envelope with the plasma membrane, which leads to release of the viral core into the cytoplasm of the cell.

As outlined below, recent research has revealed new viral entry routes, including caveolae- and lipid raft-mediated endocytosis, and their detailed characterization is underway. These investigations will lead to further understanding of general cellular processes used in the internalization of extracellular material, elucidation of viral pathogenesis as well as finding novel potential targets for antiviral therapy.

**Key messages**

- Interaction of viruses with their cell surface receptors determines the subsequent internalization events. The entry process is often controlled by specific signalling cascades.
- In addition to classical clathrin-mediated endocytosis, viruses can use several other internalization mechanisms, like those involving cell surface caveolae and lipid rafts, which direct them to distinct intracellular locations for replication.
- Viruses can be used as tools to study the complex cellular endocytic mechanisms.

## Lipid rafts: Composition and cellular function

The original view of plasma membrane structure is based on the fluid-mosaic model by Singer and Nicholson (5). They proposed that membrane proteins and lipids can freely diffuse within the plane of the bilayer. Since then, numerous studies revisiting the architecture of biological membranes have created a more complex picture. Presently, plasma membranes are considered to be laterally heterogeneous and composed of structurally and functionally distinct microdomains, including lipid rafts. Despite the fact that rafts have been extensively studied for a relatively long time, there are still various hypotheses about their nature.

The most commonly cited hypothesis (6) describes lipid rafts as relatively large (larger than 50 nm) structures, enriched with cholesterol and (glyco)sphingolipids and certain proteins. Based on this proposal, the outer leaflet of the plasma membrane rafts contains sphingomyelin and

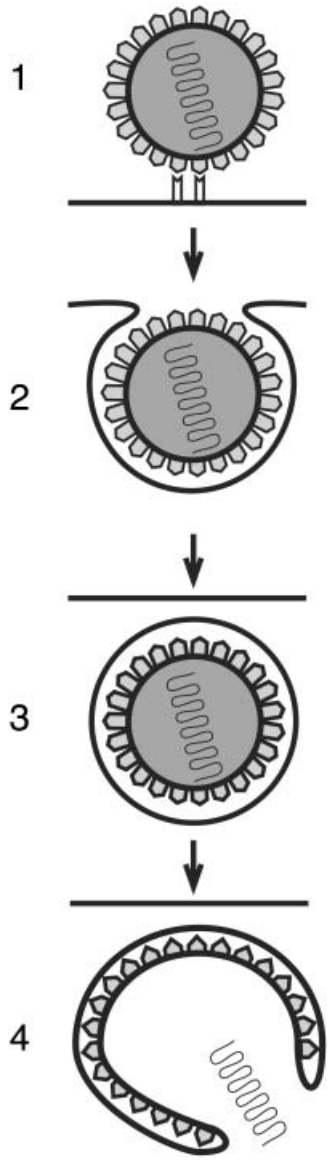


Figure 1. Schematic presentation of viral endocytosis. Virus-receptor interaction **1**) gives rise to formation of virus-containing membrane vesicles **2**), which are internalized into the host cell **3**). Subsequently, the viral core is released from these structures **4**) by a fusion of the viral envelope and the cellular vesicular membrane or by a rupture of the endocytic vesicle.

glycosphingolipids, such as ganglioside GM1 (7), while the inner leaflet contains glycerophospholipids (reviewed in (6)). The more recently published 'shell' hypothesis of lipid rafts (8) proposes that certain proteins interact with cholesterol-rich assemblies and form small shells. These shells can then, in activating conditions, fuse together and form larger rafts. Moreover, the experiments with glycosylphosphatidylinositol (GPI)-anchored proteins in living cells (9–11), suggest that pre-existing lipid assemblies are small and dynamic high density structures, which can be induced to form larger rafts.

Certain proteins, such as GPI-anchored proteins (9,12), and signalling molecules, like tyrosine kinases of the Src family (13,14) can be associated with lipid rafts. The morphologically distinct raft microdomain invaginations, with high amounts of caveolin proteins on the cytoplasmic leaflet, are termed caveolae (15). Several studies indicate that many proteins are not constitutively present in rafts but protein cross-linking with a ligand or antibodies may collect them to the raft regions (16) and induce signalling events (reviewed in (17)). The functions of rafts can be associated, for example, with cholesterol transport (reviewed in (18)), endocytosis (19), and signal transduction (reviewed in (17)).

Assembly of rafts takes place in the Golgi complex from where they move to the plasma membrane. The recycling back to the Golgi may regulate their distribution and composition (20). The raft composition is dependent especially on cellular control of cholesterol biosynthesis, uptake and deposition. Defects in these processes or in the composition and amount of rafts can result in diseases (reviewed in (21)). For example, in Alzheimer disease, raft-dependent processing of amyloid-beta-peptide, a hallmark of the illness, is impaired (22).

Traditionally, purification of lipid rafts and their components has been based on their insolubility in detergents, such as cold 1% Triton-X-100 (12) and on their low density in sucrose gradients. Therefore, lipid rafts have been considered to be detergent-resistant membranes (DRMs) (12). Also, sequestering of cholesterol with chemical compounds or oxidation of cholesterol by cholesterol oxidase may prevent raft formation (reviewed in (23)). While these approaches have been widely used to identify the protein components and to monitor raft-mediated signalling, they may result in wrong interpretations. For example, many cell components may be detergent-resistant even though they are not present in rafts (reviewed in (24)) and, moreover, rafts are not always sensitive to cholesterol depletion (25). Therefore, it remains to be seen whether some earlier described structural and functional properties of rafts must be reconsidered. Other detection methods for rafts, including characterization of determinants of protein segregation to lipid rafts with fluorescent probes (26), are required for future studies.

## Caveolae and caveosomes

Caveolae are the best characterized types of lipid rafts. They were described over 50 years ago as stable flask-shaped invaginations of the plasma membrane (27,28). Caveolae are present on the

surface of many cell types and they differ from non-caveolar lipid rafts by containing caveolin-1 as their main protein component (15,29). Caveolin-1 is a palmitylated, cholesterol-binding protein (30,31), expressed in various tissues. The caveolin gene family also includes two other members: caveolin-2, which is usually coexpressed with caveolin-1 (32,33), and caveolin-3 which is found in muscle tissue (34,35). Rapid transport of cholesterol to the cell surface is dependent on caveolin-1 expression (36) and cholesterol, in turn, is required for the existence of caveolae (15,37,38). Caveolins can oligomerize in the endoplasmic reticulum (ER) and the Golgi (39) to form a filamentous coat of cell surface caveolae (40). A pool of caveolin-1 is found in the Golgi complex (29), in caveosomes (41) and also in endosomes of clathrin-mediated endocytosis (42,43).

Originally, Parton et al. (44) demonstrated that the internalization of caveolae could be regulated by kinase inhibitors, and this was followed by another study (45) showing that the purified caveolae contain molecular components needed for regulated transport, including various signalling molecules. The later findings revealed that caveolar signalling molecules include serine/threonine protein kinase Raf-1 (46), protein kinase C-alpha (47) and protein tyrosine kinases of src-family (14). Also, endothelial nitric oxide synthase (eNOS) is targeted to caveolae via palmitylation and thus caveolae may regulate the synthesis of nitric oxide (NO) (48). Tyrosine phosphorylation of caveolin-1 (49) is important for some of these signalling events.

Because caveolae regulate several signalling cascades, mutations or defects in caveolin proteins play a role in the development of illnesses, including diabetes, cancer, cardiovascular diseases, atherosclerosis, pulmonary fibrosis, and a variety of degenerative muscular dystrophies (reviewed in (50)). Mutations in caveolin-3 gene are linked to certain hereditary forms of muscular dystrophy (51) and its expression is upregulated in the brain tissue of Alzheimer patients (52). Surprisingly, caveolin-1 knock-out mice are viable and fertile even though they exhibit a complete loss of endothelial caveolae (53). However, their life-span is shortened (54) and they suffer from pulmonary defects and vascular and hyperproliferative abnormalities related to impaired NO and calcium signalling (53).

Caveolin-1 and caveolae are partially immobile at the plasma membrane (55) and their endocytosis requires a stimulus such as a ligand binding. Caveolar fission from the cell surface to the cytoplasm is inducible by GTPases (19), like dynamin-2 (56,57), and it may also require protein kinase C (58) and actin (44,59). However, dynamin-2 is not a specific marker for caveolar fission, because it also acts in formation of clathrin-coated vesicle (60) and may, in addition, be involved in currently less well-defined endocytic mechanisms (61). The fission of vesicles is regulated by a balance of caveolin-1, cholesterol and glycosphingolipids at the plasma membrane (62). Because of the tight regulation of caveolar function, caveolae can process surface-bound ligands differentially (45). The ligands of the caveolar route involve autocrine mobility factor (63), cholera toxin (64,65), bacteria (66), viruses (67) and prions (68).

The cytoplasmic caveolae are discrete carrier vesicles that can merge with endosomal vesicles of the clathrin-mediated pathway to release their cargo in there (43) or fuse with caveosomes (41). Caveosomes are pH-neutral, caveolin-1 positive, pre-existing organelles rich in cholesterol and glycosphingolipids (41). The caveolar endocytosis, however, may not be the only route from the cell surface to caveosomes (69). From caveosomes, the ligands can be sorted to other cellular locations, such as the ER (41,70).

## Viruses that enter host cells via caveolar endocytosis and caveosomes

The endocytic routes of simian virus 40 (41,69,71,72) and echovirus 1 (73,74) to caveosomes are discussed here in detail, because their entry mechanisms have been investigated most extensively (Figures 2A, 2B). Other viruses that may utilize the caveolar entry pathway in certain cell types include polyomaviruses (75), influenza viruses (76), and coronaviruses (77). Caveolar pathway has also been associated with the endocytosis of some papillomavirus types (78) and respiratory syncytial virus (79,80).

### *Simian virus 40 (SV40)*

This non-enveloped DNA virus of the papovavirus family, can bind onto the cell surface using two different receptors, ganglioside GM1, located in lipid rafts (81), and major histocompatibility (MHC) class I molecule (82). The original studies showed that binding of SV40 to the MHC class I molecules results in the receptor clustering and redistribution with the virus to the cell surface caveolae (83,84). The virus is tightly enclosed into caveolae which are smaller in the presence of the virus (83). SV40 may recruit more caveolae from the cytoplasm and, moreover, even new caveolae may form to the site of entry (85). After 20 min, the

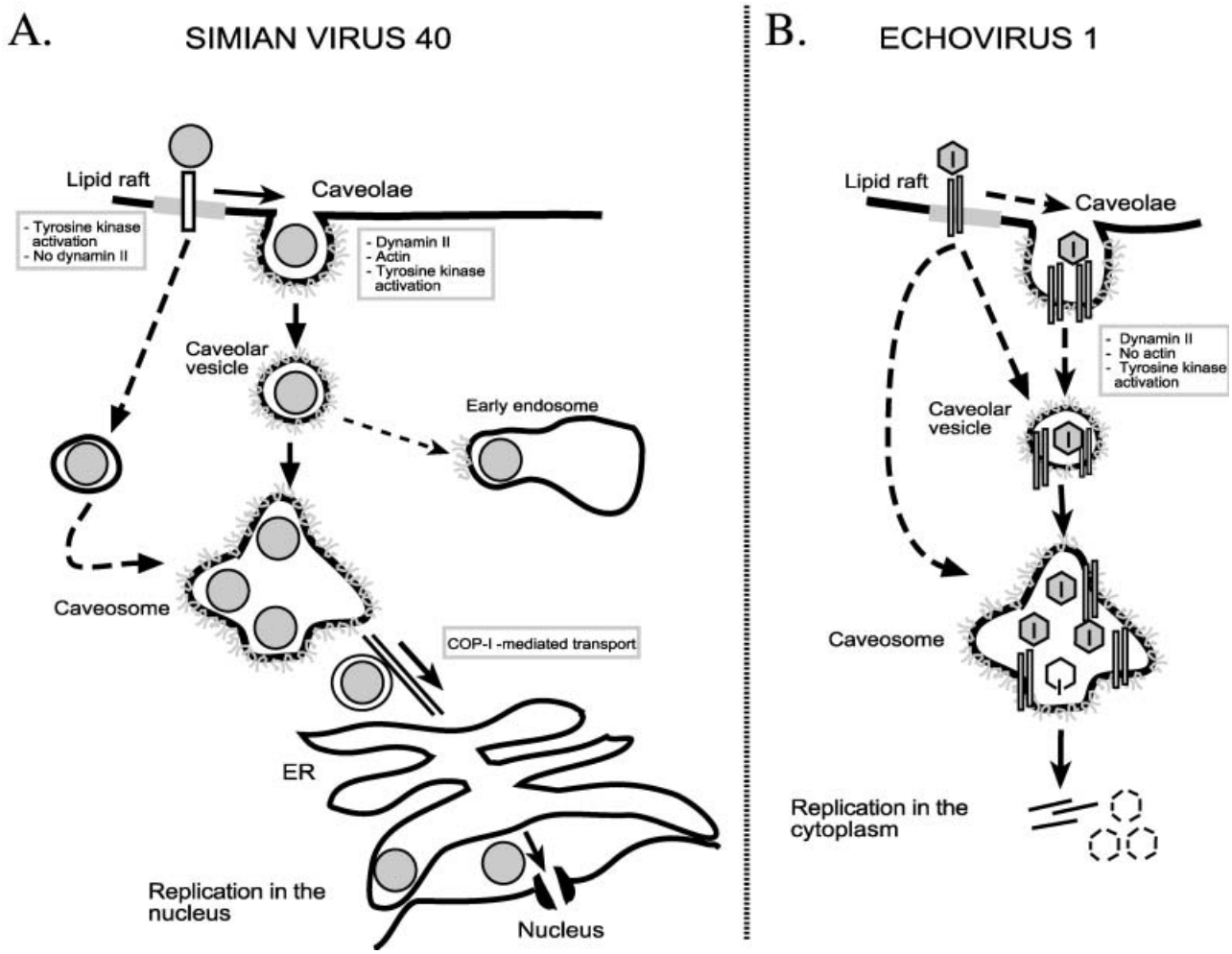


Figure 2. Endocytosis of simian virus 40 and echovirus 1 to the caveosomes. **A**. Simian virus 40 is endocytosed into caveosomes via caveolae and caveolar vesicles. In some cell types the virus can enter the caveosomes directly from lipid rafts in non-coated vesicles. Some of internalized SV40 particles can also be found in the endosomes. **B**. Echovirus 1 is internalized together with its receptor, $\alpha 2\beta 1$ integrin, into caveosomes via cell surface caveolae or by an alternative pathway, which may originate from lipid rafts and does not involve clathrin-coated pits. EV1 may remain in caveosomes prior to initiation of replication.

virus-containing caveolae invaginate from the cell membrane which is caused by a cascade of virus-induced signalling events. These involve local tyrosine kinase phosphorylation and protein kinase C activation (41,86), as well as production of phosphatidylinositol 4,5-bisphosphate (85). The signalling leads first to the transient breakdown of actin stress fibers and then to recruitment of actin to caveolae where it polymerizes again and forms patches that serve as platforms for actin tail formation (72). Tyrosine kinase phosphorylation recruits dynamin-2 transiently to the neck of caveolae. Finally, the caveolar vesicles are formed and released into the cytoplasm (72), where they associate with caveosomes. The MHC class I molecule is not endocytosed with the virus (83).

In normal conditions, a majority of SV40 particles are found in caveosomes, however, a small proportion of the virus is also trapped from caveolar vesicles into early endosomes by GTPase (guanosine 5'-triphosphate-hydrolysing enzyme) Rab5-dependent manner (43). Overexpression of a constantly active form of Rab5 results in an artificial guiding of most of the virus into the endosomes, however, the infection does not proceed (43).

In the green monkey kidney cell line CV-1, the uptake and replication of SV40 is efficiently prevented with inhibitors specific for lipid rafts and/or caveolae (71,72) and with dominant negative mutants of caveolin-1, caveolin-3 and dynamin-2 (41,72,87). Somewhat unexpectedly, SV40 was recently shown to be able to infect caveolin-1 knock-out mouse cells (69). The alternative pathway, like caveolar endocytosis of SV40, bypasses the endocytic organelles of clathrin route, is dependent on cholesterol and carries the virus into the

caveosome-like organelles in cells lacking caveolin-1. However, the alternative pathway is more rapid and does not require dynamin-2 (69). Interestingly, this pathway can also be occupied in cells that express caveolin-1. The uptake of SV40 via the alternative pathway may begin from non-caveolar lipid rafts of the plasma membrane. Then, the virus is internalized in small intracellular, uncoated vesicles to caveosome-like organelles or to caveosomes (69).

Once in the caveosomes, SV40 can remain there for several hours, after which it is sorted by tubular, caveolin-1 negative carriers that move along the microtubules to the ER (41,88). These events may involve COPI- and COPII-coated carrier vesicles (88). From the ER, SV40 enters the nucleus through the nuclear pore complexes for replication (reviewed in (89)) (Figure 2A).

A related virus, murine polyomavirus, binds also to gangliosides (GD1a, GT1b), present in lipid rafts (81,90), and depending on the target host cells, the virus is internalized via a non-caveolar, non-clathrin dependent manner (91,92) or through caveolae (75,93). From caveolae, the internalization proceeds to caveosomes and by a microtubule-dependent manner to the ER (93).

### *Echovirus 1 (EV1)*

Echovirus 1 is a member of the picornavirus family of non-enveloped, single-stranded RNA viruses. On the host cell, EV1 binds to the I domain of $\alpha 2\beta 1$ integrin, a collagen receptor (94,95). Antibodies against $\beta 2$-microglobulin can also prevent EV1 infection, but whether this molecule has a role in the virus entry is currently unknown (96). Based on cryo-electron microscopy reconstruction of EV1-$\alpha 2\beta 1$ integrin interaction, the multiple integrin heterodimers can bind to the adjacent sites of the virus capsid (97). This interaction may induce clustering of the integrin molecules (97) and result in relocation of integrins from lipid rafts to the caveolae-like structures (98). Immuno-electron microscopy of the infected cells reveals that some EV1 particles are clearly located into uncoated cell surface structures that are morphologically indistinguishable from caveolae (73). However, in live fluorescence microscopy, most EV1 particles were not detected in green fluorescent protein (GFP)-tagged caveolin 1-containing cell surface structures (74). This indicated that EV1 may, like SV40 (69), also enter the host cell caveosomes by an alternative mechanism, which could involve non-caveolar lipid rafts (Figure 2B).

Protein kinase C$\alpha$ is activated during the EV1 internalization process and tyrosine phosphorylation is required for the efficient replication cycle (74,98). The infection cycle is partially dependent on dynamin-2 and cholesterol and it can be, to some extent, inhibited by a dominant negative mutant of caveolin-3 (73,74). The requirement of the intact actin cortex in EV1 uptake depends on the cell type, since the drugs interfering the actin network had no effect on the EV1 infectivity in the green monkey kidney cell line CV-1, but actin-stabilizing agent diminished viral internalization into another cell line (74). Dominant negative mutant of caveolin-1, that inhibits SV40 entry, had no effect on EV1 internalization or infectivity, suggesting significant differences in the early entry steps between these two viruses.

After rapid internalization, intracellular EV1 is found in small, caveolin-1 positive vesicles and then already in 15 min in larger, caveolin-1 positive organelles. The large organelles were identified to be caveosomes because EV1 partially colocalized with SV40 during the uptake, the infection was blocked by lipid raft/caveolae/caveosomal inhibitors, and the virus was observed in caveosome-like structures in the EM (73,74). The uptake of EV1 into caveosomes seems to be faster than the internalization of SV40 into these structures. The $\alpha 2\beta 1$ integrin remains associated with EV1 in caveosomes (74).

EV1 has not so far been located in any other intracellular organelle like in the ER or the Golgi after the uptake into caveosomes. A microtubule-disturbing agent has no effect on EV1 infectivity, implicating that the virus is not transported further from caveosomes by a microtubule-dependent step. Since a remarkable amount of genomic viral RNA was observed in the caveosomes, these organelles could act as the final destination of the virus, and the uncoating and the release of the viral genome could be initiated there (74) (Figure 2B).

The uptake of both SV40 (41,69,72) and EV1 (73,74,98) into caveosomes appears to be caveolae/non-caveolar lipid raft-derived, tyrosine kinase-dependent and largely bypasses endosomes and lysosomes. In a recent genome-wide analysis of human kinases during SV40 endocytosis, integrin signalling was observed to control SV40 entry pathway (99). Interestingly, $\alpha 2\beta 1$ integrin-mediated signalling may also direct internalization of EV1 into caveosomes (98). However, there are some significant differences in detailed endocytic mechanisms of these viruses (e.g. the speed of virus internalization). Moreover, the caveosomal route directs SV40 and EV1 into different sites of replication in the cell; SV40 to the nucleus and EV1 to the cytoplasm. It will be relevant to find out how the alternative pathway(s) to caveosomes and further endocytic sorting of viruses from caveosomes can

affect the replication efficiency of viruses and the outcome of infection.

## Viruses that use the non-caveolar raft-mediated endocytosis

Lipid rafts may facilitate cell-surface interactions and internalization of several viruses, including avian sarcoma and leucosis virus (ASLV) (100), HIV (101), measles virus (102), certain picornaviruses (74,103–105), rotaviruses (106), and SV40 (69). For example, viruses may be transported by non-caveolar lipid raft-mediated endocytosis into caveosomes (69), as discussed in the previous section, or into endosomes, as shown with ASLV (100). However, the detailed uptake mechanisms via non-caveolar rafts are poorly characterized.

### *Enteroviruses*

Echovirus 11 (EV11), a picornavirus, which belongs to the enterovirus genus like EV1, may use lipid rafts during the internalization process (103). Decay accelerating factor (DAF), a complement regulatory protein, acts as an attachment receptor for a wide group of enteroviruses including EV11 (107). DAF belongs to GPI-anchored glycoproteins that can be located in lipid rafts (16). The uptake of the DAF-binding strain of EV11 can be inhibited with drugs interfering with cholesterol traffic, actin cytoskeleton and microtubules. Moreover, the virus has been copurified with the DRM fraction, strongly indicating the involvement of rafts in the internalization process. Caveolin-1 was present in the virus-positive raft fraction in one cell line but not detectable in another cell line, suggesting EV11 could enter alternatively via rafts/caveolae or through lipid rafts alone (103). Another DAF-utilizing enterovirus, coxsackievirus B4 (CBV4), was reported to enter via rafts to the Golgi (105). Moreover, the internalization of a related enterovirus, coxsackievirus A9 (CAV9), may be lipid raft-dependent (104). However, the role of caveolin-1 containing structures in the endocytosis of CAV9 and CBV4 remains to be determined.

### *Other viruses*

The receptors of rotavirus, non-enveloped double-stranded RNA virus, include a ganglioside GM1, integrins and a heat shock protein 70 that are associated with DRMs on the cell surface (108). Likewise, infectious rotavirus is located within lipid rafts during the entry (108) and cholesterol and dynamin-2 GTPase are required for an efficient infection. The fact that rotavirus can infect cells where caveolar or clathrin-mediated uptake routes are inhibited (106) suggests that non-caveolar lipid rafts, instead of caveolae, are used in its endocytosis.

Findings concerning the role of lipid rafts in HIV entry are still controversial. HIV-1 infection triggers lateral diffusion of specific membrane components after the virus has interacted with CD4, enabling subsequent interactions with coreceptors (101,109). These interactions are proposed to occur mainly in lipid rafts containing CD4 and result in productive infection of T cells (110,111). Also, viral entry is inhibited if membrane rafts are destroyed with cholesterol depletion before virus binding (101) and HIV entry to the brain microvascular endothelial cells by macropinocytosis is dependent on lipid rafts (112). Other studies propose that the presence of HIV-1 receptors in the rafts is not required for virus entry and that cholesterol can modulate the endocytic process independently of its ability to promote raft formation (113). However, fusion of the virus with the plasma membrane and subsequent steps of entry may require lipid rafts (114).

## Conclusions

Our knowledge of the role of lipid rafts, caveolae and caveosomes in cellular endocytic processes is rapidly increasing. From a virological point of view, it is of special interest that studies on the entry process of SV40 resulted in the identification of caveosomes, previously unrecognized cellular organelles (41). Further research has revealed that other viruses also utilize this pathway or take advantage of the non-caveolar lipid rafts. Several recent reports reveal previously unknown connections between different endocytotic pathways in the cells and viruses are one powerful tool to investigate these phenomena.

Pathogenesis of virus infections is largely dependent on the expression of specific molecules on the cell surface for virus attachment and entry. Our understanding on the viral receptors is still largely limited to the cultured cells and it can be expected that more complex interactions take place during clinical infections. The role of different entry routes in the outcome of the viral infections is poorly understood but this information can provide us with deeper insight into the initiation of clinical infections and it can give us additional tools for their prevention and treatment. It has already been shown that the entry and uncoating of some viruses (e.g. HIV and rhinoviruses) can be prevented by drugs and the future will most probably offer new examples of the clinical usefulness of the molecular information. In gene therapy, targeting of viral

vectors to correct tissues and cells is essential and our knowledge of receptors and internalization pathways will undoubtedly help to improve these methods in the future.

## References


1. Hyypiä T. Viral host cell receptors. In: Encyclopedia of Life Sciences. London: Nature Publishing Group; 2003.
2. Fingeroth JD, Weis JJ, Tedder TF, Strominger JL, Biro PA, Fearon DT. Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. Proc Natl Acad Sci U S A. 1984;81:4510–4.
3. Lentz TL, Burrage TG, Smith AL, Crick J, Tignor GH. Is the acetylcholine receptor a rabies virus receptor? Science. 1982;215:182–4.
4. Smith AE, Helenius A. How viruses enter animal cells. Science. 2004;304:237–42.
5. Singer SJ, Nicholson GL. The fluid-mosaic model of the structure of cell membranes. Science. 1972;175:720–31.
6. Simons K, Ikonen E. Functional rafts in cell membranes. Nature. 1997;387:569–72.
7. Parton RG. Ultrastructural localization of gangliosides; GM1 is concentrated in caveolae. J Histochem Cytochem. 1994;42:155–66.
8. Anderson RG, Jacobson K. A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. Science. 2002;296:1821–5.
9. Varma R, Mayor S. GPI-anchored proteins are organized in submicron domains at the cell surface. Nature. 1998;394:798–801.
10. Sharma P, Varma R, Sarasij RC, Ira, Gousset K, Krishnamoorthy G, et al. Nanoscale organization of multiple GPI-anchored proteins in living cell membranes. Cell. 2004;116:577–89.
11. Friedrichson T, Kurzchalia TV. Microdomains of GPI-anchored proteins in living cells revealed by crosslinking. Nature. 1998;394:802–5.
12. Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell. 1992;68:533–44.
13. Foster LJ, De Hoog CL, Mann M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proc Natl Acad Sci U S A. 2003;100:5813–8.
14. Sargiacomo M, Sudol M, Tang Z, Lisanti MP. Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. J Cell Biol. 1993;122:789–807.
15. Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG. Caveolin, a protein component of caveolae membrane coats. Cell. 1992;68:673–82.
16. Mayor S, Rothberg KG, Maxfield FR. Sequestration of GPI-anchored proteins in caveolae triggered by cross-linking. Science. 1994;264:1948–51.
17. Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol. 2000;1:31–9.
18. Simons K, Ikonen E. How cells handle cholesterol. Science. 2000;290:1721–6.
19. Schnitzer JE, Oh P, McIntosh DP. Role of GTP hydrolysis in fission of caveolae directly from plasma membranes. Science. 1996;274:239–42.
20. Nichols BJ, Kenworthy AK, Polishchuk RS, Lodge R, Roberts TH, Hirschberg K, et al. Rapid cycling of lipid raft markers between the cell surface and Golgi complex. J Cell Biol. 2001;153:529–41.
21. Simons K, Ehehalt R. Cholesterol, lipid rafts, and disease. J Clin Invest. 2002;110:597–603.
22. Ehehalt R, Keller P, Haass C, Thiele C, Simons K. Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. J Cell Biol. 2003;160:113–23.
23. Ikonen E, Parton RG. Caveolins and cellular cholesterol balance. Traffic. 2000;1:212–7.
24. Munro S. Lipid rafts: elusive or illusive? Cell. 2003;115:377–88.
25. Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K. Resistance of cell membranes to different detergents. Proc Natl Acad Sci U S A. 2003;100:5795–800.
26. Zacharias DA, Violin JD, Newton AC, Tsien RY. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science. 2002;296:913–6.
27. Palade G. Fine structure of blood capillaries. J Appl Physiol. 1953;24:1424.
28. Yamada E. The fine structure of the gall bladder epithelium of the mouse. J Biophys Biochem Cytol. 1955;1:455–8.
29. Kurzchalia TV, Dupree P, Parton RG, Kellner R, Virta H, Lehnert M, et al. VIP21, a 21-kD membrane protein is an integral component of trans-Golgi-network-derived transport vesicles. J Cell Biol. 1992;118:1003–14.
30. Dietzen DJ, Hastings WR, Lublin DM. Caveolin is palmitoylated on multiple cysteine residues. Palmitoylation is not necessary for localization of caveolin to caveolae. J Biol Chem. 1995;270:6838–42.
31. Murata M, Peränen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K. VIP21/caveolin is a cholesterol-binding protein. Proc Natl Acad Sci U S A. 1995;92:10339–43.
32. Scheiffele P, Verkade P, Fra AM, Virta H, Simons K, Ikonen E. Caveolin-1 and -2 in the exocytic pathway of MDCK cells. J Cell Biol. 1998;140:795–806.
33. Scherer PE, Okamoto T, Chun M, Nishimoto I, Lodish HF, Lisanti MP. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. Proc Natl Acad Sci U S A. 1996;93:131–5.
34. Parton RG, Way M, Zorzi N, Stang E. Caveolin-3 associates with developing T-tubules during muscle differentiation. J Cell Biol. 1997;136:137–54.
35. Way M, Parton RG. M-caveolin, a muscle-specific caveolin-related protein. FEBS Lett. 1995;376:108–12.
36. Smart EJ, Ying Y, Donzell WC, Anderson RG. A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. J Biol Chem. 1996;271:29427–35.
37. Hailstones D, Sleer LS, Parton RG, Stanley KK. Regulation of caveolin and caveolae by cholesterol in MDCK cells. J Lipid Res. 1998;39:369–79.
38. Chang WJ, Rothberg KG, Kamen BA, Anderson RG. Lowering the cholesterol content of MA104 cells inhibits receptor-mediated transport of folate. J Cell Biol. 1992;118:63–9.
39. Monier S, Parton RG, Vogel F, Behlke J, Henske A, Kurzchalia TV. VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. Mol Biol Cell. 1995;6:911–27.
40. Fernandez I, Ying Y, Albanesi J, Anderson RG. Mechanism of caveolin filament assembly. Proc Natl Acad Sci U S A. 2002;99:11193–8.
41. Pelkmans L, Kartenbeck J, Helenius A. Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. Nat Cell Biol. 2001;3:473–83.

42. Gagescu R, Demaurex N, Parton RG, Hunziker W, Huber LA, Gruenberg J. The recycling endosome of Madin-Darby canine kidney cells is a mildly acidic compartment rich in raft components. Mol Biol Cell. 2000;11:2775–91.
43. Pelkmans L, Burli T, Zerial M, Helenius A. Caveolin-stabilized membrane domains as multifunctional transport and sorting devices in endocytic membrane traffic. Cell. 2004;118:767–80.
44. Parton RG, Joggerst B, Simons K. Regulated internalization of caveolae. J Cell Biol. 1994;127:1199–215.
45. Schnitzer JE, McIntosh DP, Dvorak AM, Liu J, Oh P. Separation of caveolae from associated microdomains of GPI-anchored proteins. Science. 1995;269:1435–9.
46. Mineo C, James GL, Smart EJ, Anderson RG. Localization of epidermal growth factor-stimulated Ras/Raf-1 interaction to caveolae membrane. J Biol Chem. 1996;271:11930–5.
47. Mineo C, Ying YS, Chapline C, Jaken S, Anderson RG. Targeting of protein kinase Calpha to caveolae. J Cell Biol. 1998;141:601–10.
48. Garcia-Cardena G, Oh P, Liu J, Schnitzer JE, Sessa WC. Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. Proc Natl Acad Sci U S A. 1996;93:6448–53.
49. Glenney JR, Jr. Tyrosine phosphorylation of a 22-kDa protein is correlated with transformation by Rous sarcoma virus. J Biol Chem. 1989;264:20163–6.
50. Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. Physiol Rev. 2004;84:1341–79.
51. Woodman SE, Sotgia F, Galbiati F, Minetti C, Lisanti MP. Caveolinopathies: mutations in caveolin-3 cause four distinct autosomal dominant muscle diseases. Neurology. 2004;62:538–43.
52. Nishiyama K, Trapp BD, Ikezu T, Ransohoff RM, Tomita T, Iwatsubo T, et al. Caveolin-3 upregulation activates beta-secretase-mediated cleavage of the amyloid precursor protein in Alzheimer's disease. J Neurosci. 1999;19:6538–48.
53. Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, et al. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science. 2001;293:2449–52.
54. Park DS, Cohen AW, Frank PG, Razani B, Lee H, Williams TM, et al. Caveolin-1 null (-/-) mice show dramatic reductions in life span. Biochemistry. 2003;42:15124–31.
55. Mundy DI, Machleidt T, Ying YS, Anderson RG, Bloom GS. Dual control of caveolar membrane traffic by microtubules and the actin cytoskeleton. J Cell Sci. 2002;115:4327–39.
56. Henley JR, Krueger EW, Oswald BJ, McNiven MA. Dynamin-mediated internalization of caveolae. J Cell Biol. 1998;141:85–99.
57. Oh P, McIntosh DP, Schnitzer JE. Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. J Cell Biol. 1998;141:101–14.
58. Smart EJ, Foster DC, Ying YS, Kamen BA, Anderson RG. Protein kinase C activators inhibit receptor-mediated potocytosis by preventing internalization of caveolae. J Cell Biol. 1994;124:307–13.
59. Conrad PA, Smart EJ, Ying YS, Anderson RG, Bloom GS. Caveolin cycles between plasma membrane caveolae and the Golgi complex by microtubule-dependent and microtubule-independent steps. J Cell Biol. 1995;131:1421–33.
60. Damke H, Baba T, Warnock DE, Schmid SL. Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. J Cell Biol. 1994;127:915–34.
61. Lamaze C, Dujeancourt A, Baba T, Lo CG, Benmerah A, Dautry-Varsat A. Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. Mol Cell. 2001;7:661–71.
62. Sharma DK, Brown JC, Choudhury A, Peterson TE, Holicky E, Marks DL, et al. Selective stimulation of caveolar endocytosis by glycosphingolipids and cholesterol. Mol Biol Cell. 2004;15:3114–22.
63. Benlimame N, Le PU, Nabi IR. Localization of autocrine motility factor receptor to caveolae and clathrin-independent internalization of its ligand to smooth endoplasmic reticulum. Mol Biol Cell. 1998;9:1773–86.
64. Montesano R, Roth J, Robert A, Orci L. Non-coated membrane invaginations are involved in binding and internalization of cholera and tetanus toxins. Nature. 1982;296:651–3.
65. Nichols BJ. A distinct class of endosome mediates clathrin-independent endocytosis to the Golgi complex. Nat Cell Biol. 2002;4:374–8.
66. Shin JS, Gao Z, Abraham SN. Involvement of cellular caveolae in bacterial entry into mast cells. Science. 2000;289:785–8.
67. Pelkmans L, Helenius A. Endocytosis via caveolae. Traffic. 2002;3:311–20.
68. Peters PJ, Mironov A, Jr. Peretz D, van Donselaar E, Leclerc E, Erpel S, et al. Trafficking of prion proteins through a caveolae-mediated endosomal pathway. J Cell Biol. 2003;162:703–17.
69. Damm EM, Pelkmans L, Kartenbeck J, Mezzacasa A, Kurzchalia T, Helenius A. Clathrin- and caveolin-1-independent endocytosis: entry of simian virus 40 into cells devoid of caveolae. J Cell Biol. 2005;168: 477–88.
70. Le PU, Nabi IR. Distinct caveolae-mediated endocytic pathways target the Golgi apparatus and the endoplasmic reticulum. J Cell Sci. 2003;116:1059–71.
71. Anderson HA, Chen Y, Norkin LC. Bound simian virus 40 translocates to caveolin-enriched membrane domains, and its entry is inhibited by drugs that selectively disrupt caveolae. Mol Biol Cell. 1996;7:1825–34.
72. Pelkmans L, Puntener D, Helenius A. Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. Science. 2002;296: 535–9.
73. Marjomäki V, Pietiäinen V, Matilainen H, Upla P, Ivaska J, Nissinen L, et al. Internalization of echovirus 1 in caveolae. J Virol. 2002;76:1856–65.
74. Pietiäinen V, Marjomäki V, Upla P, Pelkmans L, Helenius A, Hyypiä T. Echovirus 1 endocytosis into caveosomes requires lipid rafts, dynamin II, and signaling events. Mol Biol Cell. 2004;15:4911–25.
75. Richterova Z, Liebl D, Horak M, Palkova Z, Stokrova J, Hozak P, et al. Caveolae are involved in the trafficking of mouse polyomavirus virions and artificial VP1 pseudocapsids toward cell nuclei. J Virol. 2001;75:10880–91.
76. Nunes-Correia I, Eulalio A, Nir S, Pedroso de Lima MC. Caveolae as an additional route for influenza virus endocytosis in MDCK cells. Cell Mol Biol Lett. 2004;9: 47–60.
77. Nomura R, Kiyota A, Suzaki E, Kataoka K, Ohe Y, Miyamoto K, et al. Human coronavirus 229E binds to CD13 in rafts and enters the cell through caveolae. J Virol. 2004;78:8701–8.

78. Bousarghin L, Touze A, Sizaret PY, Coursaget P. Human papillomavirus types 16, 31, and 58 use different endocytosis pathways to enter cells. J Virol. 2003;77:3846–50.
79. Brown G, Jeffree CE, McDonald T, Rixon HW, Aitken JD, Sugrue RJ. Analysis of the interaction between respiratory syncytial virus and lipid-rafts in Hep2 cells during infection. Virology. 2004;327:175–85.
80. Werling D, Hope JC, Chaplin P, Collins RA, Taylor G, Howard CJ. Involvement of caveolae in the uptake of respiratory syncytial virus antigen by dendritic cells. J Leukoc Biol. 1999;66:50–8.
81. Tsai B, Gilbert JM, Stehle T, Lencer W, Benjamin TL, Rapoport TA. Gangliosides are receptors for murine polyoma virus and SV40. EMBO J. 2003;22:4346–55.
82. Bernacchi S, Mueller G, Langowski J, Waldeck W. Characterization of simian virus 40 on its infectious entry pathway in cells using fluorescence correlation spectroscopy. Biochem Soc Trans. 2004;32:746–9.
83. Anderson HA, Chen Y, Norkin LC. MHC class I molecules are enriched in caveolae but do not enter with simian virus 40. J Gen Virol. 1998;79:1469–77.
84. Stang E, Kartenbeck J, Parton RG. Major histocompatibility complex class I molecules mediate association of SV40 with caveolae. Mol Biol Cell. 1997;8:47–57.
85. Pelkmans L, Helenius A. Insider information: what viruses tell us about endocytosis. Curr Opin Cell Biol. 2003;15:414–22.
86. Dangoria NS, Breau WC, Anderson HA, Cishek DM, Norkin LC. Extracellular simian virus 40 induces an ERK/MAP kinase-independent signalling pathway that activates primary response genes and promotes virus entry. J Gen Virol. 1996;77:2173–82.
87. Roy S, Luetterforst R, Harding A, Apolloni A, Etheridge M, Stang E, et al. Dominant-negative caveolin inhibits H-Ras function by disrupting cholesterol-rich plasma membrane domains. Nat Cell Biol. 1999;1:98–105.
88. Richards AA, Stang E, Pepperkok R, Parton RG. Inhibitors of COP-mediated transport and cholera toxin action inhibit simian virus 40 infection. Mol Biol Cell. 2002;13:1750–64.
89. Kasamatsu H, Nakanishi A. How do animal DNA viruses get to the nucleus? Annu Rev Microbiol. 1998;52:627–86.
90. Smith AE, Lilie H, Helenius A. Ganglioside-dependent cell attachment and endocytosis of murine polyomavirus-like particles. FEBS Lett. 2003;555:199–203.
91. Gilbert JM, Goldberg IG, Benjamin TL. Cell penetration and trafficking of polyomavirus. J Virol. 2003;77:2615–22.
92. Gilbert JM, Benjamin TL. Early steps of polyomavirus entry into cells. J Virol. 2000;74:8582–8.
93. Gilbert J, Benjamin T. Uptake pathway of polyomavirus via ganglioside GD1a. J Virol. 2004;78:12259–67.
94. Bergelson JM, Shepley MP, Chan BM, Hemler ME, Finberg RW. Identification of the integrin VLA-2 as a receptor for echovirus 1. Science. 1992;255:1718–20.
95. Bergelson JM, St John NF, Kawaguchi S, Pasqualini R, Berdichevsky F, Hemler ME, et al. The I domain is essential for echovirus 1 interaction with VLA-2. Cell Adhes Commun. 1994;2:455–64.
96. Ward T, Powell RM, Pipkin PA, Evans DJ, Minor PD, Almond JW. Role for beta2-microglobulin in echovirus infection of rhabdomyosarcoma cells. J Virol. 1998;72:5360–5.
97. Xing L, Huhtala M, Pietiäinen V, Käpyla J, Vuorinen K, Marjomäki V, et al. Structural and functional analysis of integrin alpha2I domain interaction with echovirus 1. J Biol Chem. 2004;279:11632–8.
98. Upla P, Marjomäki V, Kankaanpää P, Ivaska J, Hyypiä T, Van Der Goot FG, et al. Clustering induces a lateral redistribution of alpha 2 beta 1 integrin from membrane rafts to caveolae and subsequent protein kinase C-dependent internalization. Mol Biol Cell. 2004;15:625–36.
99. Pelkmans L, Fava E, Grabner H, Hannus M, Habermann B, Krausz E, et al. Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. Nature. 2005.
100. Narayan S, Barnard RJ, Young JA. Two retroviral entry pathways distinguished by lipid raft association of the viral receptor and differences in viral infectivity. J Virol. 2003;77:1977–83.
101. Manes S, del Real G, Lacalle RA, Lucas P, Gomez-Mouton C, Sanchez-Palomino S, et al. Membrane raft microdomains mediate lateral assemblies required for HIV-1 infection. EMBO Rep. 2000;1:190–6.
102. Avota E, Muller N, Klett M, Schneider-Schaulies S. Measles virus interacts with and alters signal transduction in T-cell lipid rafts. J Virol. 2004;78:9552–9.
103. Stuart AD, Eustace HE, McKee TA, Brown TD. A novel cell entry pathway for a DAF-using human enterovirus is dependent on lipid rafts. J Virol. 2002;76:9307–22.
104. Triantafilou K, Triantafilou M. Lipid raft microdomains: key sites for Coxsackievirus A9 infectious cycle. Virology. 2003;317:128–35.
105. Triantafilou K, Triantafilou M. Lipid-raft-dependent Coxsackievirus B4 internalization and rapid targeting to the Golgi. Virology. 2004;326:6–19.
106. Sanchez-San Martin C, Lopez T, Arias CF, Lopez S. Characterization of rotavirus cell entry. J Virol. 2004;78:2310–8.
107. Ward T, Pipkin PA, Clarkson NA, Stone DM, Minor PD, Almond JW. Decay-accelerating factor CD55 is identified as the receptor for echovirus 7 using CELICS, a rapid immuno-focal cloning method. EMBO J. 1994;13:5070–4.
108. Isa P, Realpe M, Romero P, Lopez S, Arias CF. Rotavirus RRV associates with lipid membrane microdomains during cell entry. Virology. 2004;322:370–81.
109. Nisole S, Krust B, Hovanessian AG. Anchorage of HIV on permissive cells leads to coaggregation of viral particles with surface nucleolin at membrane raft microdomains. Exp Cell Res. 2002;276:155–73.
110. Popik W, Alce TM, Au WC. Human immunodeficiency virus type 1 uses lipid raft-colocalized CD4 and chemokine receptors for productive entry into CD4(+) T cells. J Virol. 2002;76:4709–22.
111. Del Real G, Jimenez-Baranda S, Lacalle RA, Mira E, Lucas P, Gomez-Mouton C, et al. Blocking of HIV-1 infection by targeting CD4 to nonraft membrane domains. J Exp Med. 2002;196:293–301.
112. Liu NQ, Lossinsky AS, Popik W, Li X, Gujuluva C, Kriederman B, et al. Human immunodeficiency virus type 1 enters brain microvascular endothelia by macropinocytosis dependent on lipid rafts and the mitogen-activated protein kinase signaling pathway. J Virol. 2002;76:6689–700.
113. Percherancier Y, Lagane B, Planchenault T, Staropoli I, Altmeyer R, Virelizier JL, et al. HIV-1 entry into T-cells is not dependent on CD4 and CCR5 localization to sphingolipid-enriched, detergent-resistant, raft membrane domains. J Biol Chem. 2003;278:3153–61.
114. Popik W, Alce TM. CD4 receptor localized to non-raft membrane microdomains supports HIV-1 entry. Identification of a novel raft localization marker in CD4. J Biol Chem. 2004;279:704–12.