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Cell Entry of Enveloped Viruses

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ABSTRACT

Enveloped viruses penetrate their cell targets following the merging of their membrane with that of the cell. This fusion process is catalyzed by one or several viral glycoproteins incorporated on the membrane of the virus. These envelope glycoproteins (EnvGP) evolved in order to combine two features. First, they acquired a domain to bind to a specific cellular protein, named "receptor." Second, they developed, with the help of cellular proteins, a function of finely controlled fusion to optimize the replication and preserve the integrity of the cell, specific to the genus of the virus. Following the activation of the EnvGP either by binding to their receptors and/or sometimes the acid pH of the endosomes, many changes of conformation permit ultimately the action of a specific hydrophobic domain, the fusion peptide, which destabilizes the cell membrane and leads to the opening of the lipidic membrane. The comprehension of these mechanisms is essential to develop medicines of the therapeutic class of entry inhibitor like enfuvirtide (Fuzeon) against human immunodeficiency virus (HIV). In this chapter, we will summarize the different envelope glycoprotein structures that viruses develop to achieve membrane fusion and the entry of the virus. We will describe the different entry pathways and cellular proteins that viruses have subverted to allow infection of the cell and the receptors that are used. Finally, we will illustrate more precisely the recent discoveries that have been made within the field of the entry process, with a focus on the use of pseudoparticles. These pseudoparticles are suitable for high-throughput screenings that help in the development of natural or artificial inhibitors as new therapeutics of the class of entry inhibitors.

I. INTRODUCTION

Enveloped viruses have a core incorporating the genetic material of the virus surrounded by a lipidic membrane acquired from the cells they bud from. Their genetic material enters target cells following the merging of their membrane with the membrane of the cells at either the plasma membrane or the membrane of another internal compartment. The process of membrane merging is called

membrane fusion, which preserves the integrity of the cell membrane. The fusion of two separate lipid bilayers in a nonaqueous environment first requires that they come into close contact. This is followed by an intermediate stage characterized by the merger of only the closest contacting monolayer, a process called hemifusion. Third, the fully completed fusion results in whole bilayer merging, followed by the opening of the pore. To achieve this process, the envelope glycoproteins (EnvGP) have evolved in order to combine two features. On the one hand, they acquired a domain to bind to a specific cellular protein, named "receptor." On the other hand, they developed in a different manner, according to the genus of the virus, a function of fusion that allows the destabilization of the membrane and the opening of a pore through which the genetic material will enter the cell.

Despite three different classes of fusion protein having been described so far, three common main steps are described for achieving the pre- to post-conformational changes. The first one, after EnvGP activation upon receptor binding or acidification of the endosomal compartment, exposes the fusion peptide that is projected toward the top of the glycoprotein, allowing the initial interaction with the target membrane. The second one is the folding back of the C-terminal region onto a trimeric N-terminal region that leads to the formation of a postfusion protein structure. The final and third step also requires further refolding of the membrane proximal and transmembrane regions in order to obtain a full-length postfusion structure where both membrane anchors (fusion peptide and tm domains) are present in the same membrane.

The viral glycoprotein-induced fusion must be controlled to allow the virus to leave the cell, to prevent the aggregation of the viruses, and to release genetic material next to a compartment that will permit the continuity of the infectious cycle. This regulation is executed principally by the inactivation or the masking of the fusion machinery that directly disorganizes the lipid bilayer. On one hand, most of these EnvGP are synthesized as a precursor that requires a cleavage by a cellular protease (like furin) and prepares the molecules for the subsequent necessary changes of conformation for the fusion process. On the other hand, the activation of the conformational changes is induced by interaction with the receptor and/or the action of the pH (that protonates some amino acids), modifying the interactions of the EnvGP and their structure. In addition, some proteases or other enzymes are necessary to achieve some complementary priming of the EnvGP to make it competent for fusion (such as cathepsin B and L).

Two types of fusion mechanisms can occur, namely, pH independent and pH dependent. In the first case, the recognition between virus and receptor directly triggers conformational changes in the EnvGP that leads to the direct fusion between the two membranes (viral and plasma) and to the liberation of the viral genetic material. This activation of EnvGP at neutral pH allows the fusion *in vitro* and *in vivo* of EnvGP-expressing cells with receptor-expressing cells. This fusion leads to the merging of cell cytoplasms and to the generation of

multinucleated cells called syncytia. In the second case, for pH-dependent fusion, the interaction between the EnvGP and the receptor leads to the obligatory endocytosis of the virus–receptor complex, and the acidification of endosomes triggers conformational changes in the EnvGP. For the pH-dependent virus, such a fusion can be reproduced in cell culture *in vitro* or in a liposome–virus fusion assay in the tube after decreasing the pH, but cannot occur *in vivo*.

Research during the last few years has greatly advanced our understanding of the cell surface receptors for viruses and has provided many surprising insights. These advances were achieved largely by identification and molecular cloning of the cell surface or cytoplasmic proteins that have been subverted for use as viral receptors or cofactor, and by parallel advances in studies of the viral EnvGP that bind to the receptors.

Another key area of new insights concerns the physical–chemical process of viral adsorption and of pulling the virus closely onto the cellular membrane. Indeed, adsorption is a severely limiting step in infections of cultured cells, and the initial attachment often does not involve the receptors that ultimately mediate infections (Andreadis *et al.*, 2000; Guibinga *et al.*, 2002; Pizzato *et al.*, 1999, 2001; Ugolini *et al.*, 1999). Thus, we need to distinguish cell surface molecules such as heparan sulfate proteoglycans, DC-SIGN, or integrins that can enhance infections by concentrating retroviruses onto cells (Bounou *et al.*, 2002; Geijtenbeek *et al.*, 2000; Jinno-Oue *et al.*, 2001; Mondor *et al.*, 1998; Pohlmann *et al.*, 2001; Saphire *et al.*, 2001) from authentic receptors that induce conformational changes in EnvGP that are a prerequisite for fusion of the viral and cellular membranes. In contrast to other cell surface components such as lectins or proteoglycans that influence infections indirectly by enhancing virus adsorption onto specific cells, the true receptors induce conformational changes in the viral EnvGP that are essential for infection. However, it appears that more and more intracellular proteins have roles in controlling viral host ranges, and the proteins involved in traffic of intracellular vesicles like endosomes, play a critical role in entry. Therefore, proteins from pathways specifically characterized might be considered as a cofactor as their role is not to mediate direct contact with EnvGP but is crucial for entry. In addition, some viruses are able to use more than one endocytic pathway and the cellular proteins involved thus direct the virus to the entry door beneficial for the virus under a particular condition.

Pseudoparticles are retroviridae cores which incorporate heterogeneous envelope protein from a different virus, possibly of a different family. Their entry process mimics precisely that of the wild-type viruses from which the envelope glycoprotein was derived. They represent a useful tool to study molecular processes of envelope viruses as they are very flexible, allowing the analysis of numerous mutants. Moreover, they allow a precise measurement of the infectivity that depends on the envelope glycoprotein for the entry step and they allow the establishment of virus–liposome fusion assays.

We will first introduce envelope architecture and structure of the viral fusion machineries that have been developed to achieve the fusion and entry steps. Despite many differences of structure, they share a common refolding process that activates different fusion domains found in most fusion proteins. We will then illustrate the different fusion regulation processes that have been developed by viruses to lead to functional virions, both in terms of cleavage activation of fusion proteins during exit and in terms of activation during binding and endocytosis. Despite some differences between distinct players, some common principles can be proposed for all fusion processes. We will illustrate the molecular details characterizing the maturation of the different fusion proteins, defined by the following three characteristics: the cleavage of an envelope protein precursor, the presence and triggering of the exposition of a fusion peptide, and an association as a trimeric complex association in its active fusion conformation. The progression of these structural rearrangements slows down the kinetic barrier between hemifusion and fusion-pore formation. In a second part, we will present the different entry pathways and cell proteins that are used by viruses to infect cells. Viruses have emerged as valuable tools for the study of endocytic mechanisms. These properties have been crucial for the development of pseudoparticles for their use in terms of vector for gene therapy and for their use in terms of tool for receptor cloning, transgenesis, or transduction. Finally, we will give examples of strategies that have been developed *in vivo* and *in vitro* to inhibit the entry step of the enveloped viruses which have led, in the case of HIV, to the development of inhibitors that are used in the clinic.

II. ARCHITECTURE AND STRUCTURE OF THE VIRAL FUSION MACHINERY

A. Membrane fusion according to the stalk-pore model

EnvGP are responsible for bringing the membranes closer, triggering the link and the destabilization of the outer leaflet, the merging of the whole membrane, and the opening of a pore through the cell and virus membrane to allow the core to enter the cytoplasm of the cell. The hypothesis of the pore model in viral membrane fusion mechanism is supported by experimental results. The first evidence for a hemifusion intermediate was achieved by studying influenza virus entry that occurs after the hemagglutinin glycoprotein binding to the host cell. The substitution of the hemagglutinin transmembrane domain by a glycosylphosphatidylinositol (GPI) revealed the importance of the transmembrane region for the fusion pore opening and expansion. Hemifusion structures are connections between outer leaflets of apposed membranes, whereas the inner leaflets remain distinct. This is a transient structure that either dissociates or

gives rise to the fusion pore (Chernomordik and Kozlov, 2008). Interestingly, the helix breaker residues within the tm domain are critical for the fusogenicity of different retroviral Env, such as HIV (Owens *et al.*, 1994) and Mo-MLV (Taylor and Sanders, 1999), being important for both the hemifusion and pore opening step of the fusion process. In addition, hemifusion intermediate has been detected in the case of HIV Env-mediated fusion (Munoz-Barroso *et al.*, 1998) by using peptide inhibitors that target a prefusion or prehairpin structure such as HIV-1 gp41 T-20. Once the pore is formed, it allows a connection between two compartments initially separated by the apposed membranes.

The ability of the membrane to hemifuse and develop a fusion pore has been found to depend on the lipid microdomain composition, for example, cholesterol (Chernomordik and Kozlov, 2003). Indeed, a potential lipid dependence of virus entry processes was first deduced from experiments on influenza virus, implying a role for detergent-resistant lipidic microdomain (Takeda *et al.*, 2003). For retroviruses, the tm palmitoylations which contribute to the Env localization in detergent-resistant lipidic microdomain domains (Li *et al.*, 2002) indirectly influence the fusion process (Ochsenbauer-Jambor *et al.*, 2001). As an alternative to the lipidic pore hypothesis, a direct fusion mechanism has also been proposed. The fusion pore is a full proteic channel-like structure dependent only on the transmembrane domains of the glycoproteins. In this model, the pore is opened by the joining of two hemipores located on each membrane (Chernomordik and Kozlov, 2005, 2008).

After fusion pore opening and enlargement (Melikyan *et al.*, 2005), the genetic material enters the cytoplasm of the cell, instigating the virus cell cycle.

B. pH-dependent and -independent molecular switches

The fusion of the viruses that enter directly at membrane plasmic level (as with the paramyxoviruses and most retroviruses) is triggered by the activation of the viral envelope protein at neutral pH. In this case, only the binding of the virus to its receptor activates the fusogenic potential of the complexes of EnvGP. This mechanism is pH-independent. In the case of retroviruses, the binding of the surface subunit (SU) to its receptor induces conformational changes not only in itself but also in the TM with which it interacts, thus inducing fusion. This activation at neutral pH permits the envelope glycoprotein of pH-independent viruses, in certain experimental conditions or *in vivo*, to induce the fusion between the cells expressing the envelope glycoprotein and the cells expressing the receptor (Harrison, 2008; Kielian and Rey, 2006; Weissenhorn *et al.*, 2007). This intercellular fusion, by merging their plasma membranes, places the cell cytoplasms in continuity, and one or more cells become giant multinucleated cells named syncytia.

In contrast, the fusion of Fig. 4.1 most other viruses depends strictly on their internalization into one of the numerous endocytic pathways such as the clathrin-dependent, clathrin-independent, and caveolae-independent, as well as the macropinocytosis (Vaccinia virus) (Mercer and Helenius, 2008) or the phagocytose (as recently described for Equine herpesvirus 1 virus (Frampton *et al.*, 2007) and the HIV virus (Trujillo *et al.*, 2007), although in this case not resulting in a productive infection; Marsh and Helenius, 2006; Sieczkarski and Whittaker, 2002; Figure 4.1). As described so far, the enveloped viruses that use these itineraries have fusion reactions that require exposure to a moderately acid pH in the different endocytosis vesicles (pH-dependent viruses). Classical examples of viruses that fuse at low pH with the membrane of endosomes or artificial membranes in the test tube include the influenza orthomyxovirus, the dengue or the tick-borne encephalitis (TBEV) flaviviruses, and the Semliki forest alphavirus (SFV) (Harrison, 2008; Kielian and Rey, 2006; Roche *et al.*, 2008; Weissenhorn *et al.*, 2007).

Interestingly, an intermediate mechanism has been described for two retroviruses, ASLS and JSRV. The proposed mechanism of ASLV virion entry occurs in two steps involving a receptor-priming step that induces Env conformational changes, thus allowing the Env to become sensitive to the action of acid pH (Mothes *et al.*, 2000). This hybrid mechanism does not lead to cell–cell fusion *in vivo*. JSRV also uses receptor priming for fusion activation of JSRV Env at a low pH, but the mechanism differs slightly to ASLV, requiring dynamin-associated endocytosis (Bertrand *et al.*, 2008).

So far, many different endocytosis pathways have been described (Marsh and Helenius, 2006; Mercer and Helenius, 2009; Mercer *et al.*, 2010b) as being used by both pH-dependent and pH-independent viruses. However, reinvestigations of these entry pathways are clearly needed for many pH-independent viruses that were originally thought not to rely on endocytosis. For example, Nipah paramyxoviruses induce fusion between cells at neutral pH and were considered as a pH-independent virus not reliant on endocytosis for entry. However, recently, it was proposed that Nipah viruses (NiVs) use macropinocytosis for entry (Pernet *et al.*, 2009). It should be noted that viruses that use a pH-independent mechanism of activation of EnvGP may still enter the cell by endocytosis without any imperative requirement for acidification activation of EnvGP in the endosomes.

Clathrin-dependent endocytosis is the best-characterized pathway of entry and is the major itinerary of entry for pH-dependent viruses. This process is initiated by the formation of the characteristic invaginations of membrane, known as clathrin-coated pits (CCP) (Fig. 4.1). The CCP assembly takes place on the internal face of the plasma membrane following a signal induced by the activation of a receptor. This clathrin-dependent method of internalization is used by the Semliki forest and Sindbis alphaviruses, the rubella rubivirus, and the

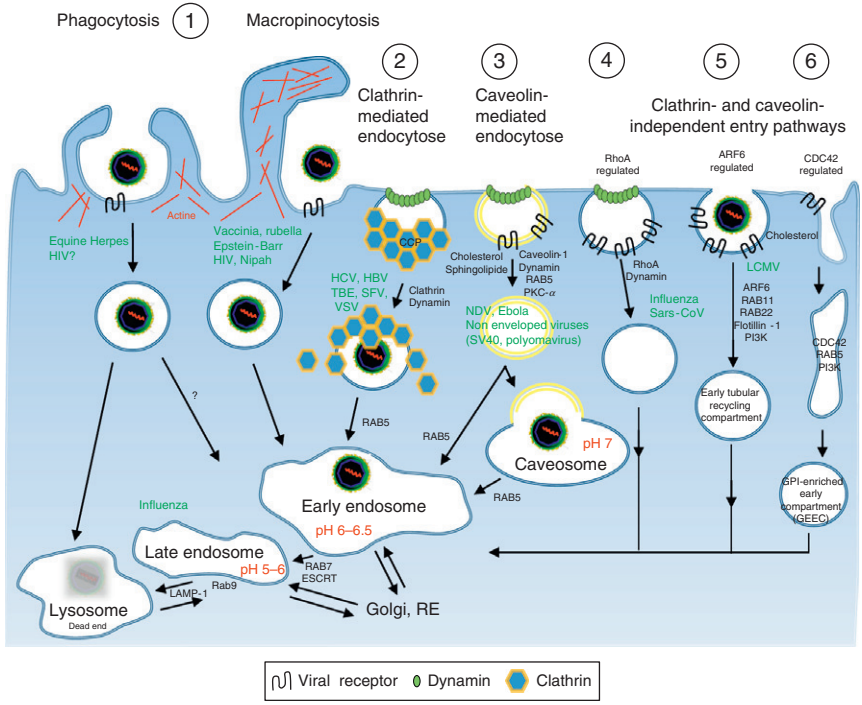

Figure 4.1. Viruses exploit different endocytosis pathways to enter host cells. Multiple mechanisms have been defined as pinocytic, that is, they are involved in the uptake of fluids, solutes, and small particles. These include clathrin-mediated, macropinocytosis, caveolar/raft-mediated mechanisms, as well as several novel mechanisms. Large particles are taken up by phagocytosis, a process restricted to a few cell types. Though poorly documented, the entry of some viruses can be mediated by numerous cargoes which can be endocytosed by mechanisms that are independent of the clathrin coat protein and the fission GTPase, dynamin. These pathways include RhoA- (IL-2 pathway), ARF6-, and CDC42-regulated pathway (GEEC pathway). After binding of the particles to the cell surface entry receptor, genetic material is delivered into the cytoplasm of the cell via a specific endocytosis pathway. Recently, macropinocytosis has emerged as an important entry pathway for viruses (1). The entry of most pH-dependent viruses is mediated by the use of clathrine-coated endocytic vesicles (2). Other viruses penetrate host cells via the formation of vesicles covered by caveola molecule (3). Using this pathway, the viral particles are targeted to neutral pH caveosomes or to early endosomes with moderately acid pH. Alternately, the virus can use non-clathrin, non-caveolin-dependent pathways, both dynamin-dependent or independent (4, 5, 6). These pathways are differentiated by the implication of different GTPases (RhoA, CDC42, or ARF6) and their different compositions (cholesterol, flotillin, TEM, etc).

Hantaan hantavirus, for example (DeTulleo and Kirchhausen, 1998; Helenius *et al.*, 1980; Jin *et al.*, 2002; Kee *et al.*, 2004). Macropinocytosis is another endocytosis pathway utilized by viruses belonging to vaccinia, adeno, picorna, and other virus families. Macropinocytosis is an endocytic mechanism normally involved in fluid uptake induced by growth factors, phorbol ester. However, the binding of virus particles can also activate signaling pathways that trigger actin-mediated membrane ruffling and blebbing. This is followed by the formation of large vacuoles (macropinosomes) at the plasma membrane, internalization of virus particles, and penetration by the viruses or their capsids into the cytosol through the limiting membrane of the macropinosomes (Mayor and Pagano, 2007; Mercer and Helenius, 2009). A variety of evidences suggest that macropinocytosis can be a mechanism for HIV-1 and Epstein-Barr herpes virus entry in some primary cells or cell lines (Marechal *et al.*, 2001; Miller and Hutt-Fletcher, 1992). Based on the more defined criteria of macropinocytosis (Mercer and Helenius, 2009), these conclusions are probably premature and may depend on the experimental conditions that use very concentrated viruses which may influence the utilization of less specific pathways. It should be noted that different strains of the same virus can elicit dramatically different responses in host cells during entry, and different macropinocytic mechanisms are possible in the same cell line through subtle differences in the activating ligand (Mercer *et al.*, 2010a).

An additional endocytosis pathway, whilst badly defined, is known as a clathrin- and caveola-independent pathway. The dependence on different GTPase and cargo proteins (dynamin, Rho, CDC42, ARF6, etc.) defines this endocytosis pathway. Several viruses are using this clathrin- and caveola-independent pathway, including the influenza orthomyxovirus (as a second possible pathway), the SARS-CoV coronavirus, the lymphocytic choriomeningitis arenavirus (LCMV), and picornaviruses (Madshus *et al.*, 1987; Matlin *et al.*, 1981; Wang *et al.*, 2008). Finally, other vesicles (characterized by caveolin or caveosome, with a high concentration in cholesterol and sphingolipids) are clathrin-independent but dynamin-dependent and do not have acid compartments before their merging with early endosomes (Mayor and Pagano, 2007). The nonenveloped SV40 virus and some human enteroviruses are the prototypes of the pH-independent viruses that use this endocytosis pathway. So far, only two enveloped viruses have been described to use this pathway, the Newcastle disease paramyxovirus (NDV) (Cantin *et al.*, 2007) and Ebola virus (Empig and Goldsmith, 2002; Sanchez, 2007). Most viruses have been shown to use only one entry pathway; however, there are more and more recent examples that indicate that some viruses use multiple endocytosis pathways to enter their target cells (influenza, HIV). For example, Ebola viruses are known to enter cells by clathrin-mediated endocytosis (Sanchez, 2007), but lipid raft-associated, caveolin-mediated endocytosis has also been proposed as a mechanism of Ebola virus uptake (Empig and Goldsmith, 2002).

In terms of the activation process of the membrane fusion and the need for particular microdomains, it is not always clear why the viruses are using so many different pathways. It is possible that the viruses that evolved to fuse intracellularly have a selective advantage to release their genome to specific intracellular sites that will allow the rapid and efficient establishment of the infectious cycle. One selective advantage could also be the requirement for particular lipid microdomains, like those enriched in cholesterol. Indeed, it was shown that contrary to the class II flavivirus dengue virus and the yellow fever virus, the E1 fusion protein from Semliki forest virus (SFV) binds cholesterol which explains its dependence for cholesterol and compartment containing cholesterol (Umashankar *et al.*, 2008). For other viruses, the dependence on cholesterol is linked to bulk effects on membrane fluidity and the maintenance of particular microdomains where receptors are located and where they have some particular diffusion.

C. The entry process can be achieved by different number of EnvGP

In addition to these dichotomies of pH-independent or pH-dependent fusion process, of plasma or endosomes membrane fusion, the mechanisms of activation of the fusion proteins of the different enveloped viruses are very diverse. Some reactions of fusion are triggered by the interaction of one envelope glycoprotein with a unique receptor. One example is the interaction of the envelope glycoprotein SU–TM of the γ-retrovirus with the multitransmembrane receptor (Table 4.1). In another case, one unique envelope glycoprotein can interact with several receptors. For example, the fusion of HIV-1 is triggered by the sequential interaction of the SU gp120 with the CD4 receptor, followed by interaction with a coreceptor such as CCR5 or CXCR4, chimiokine receptors of seven transmembrane domains (Hunter, 1997). Previously, it was believed that binding to receptors directly triggered a series of conformational changes in the viral EnvGP culminating in fusion of the viral and cellular membranes. However, new evidence suggests that γ-retroviral association with receptors triggers an obligatory cooperative interaction or cross-talk between EnvGP on the viral surface for HIV and MLV (Tailor *et al.*, 2003). If this intermediate step is prevented, infection fails. Conversely, in several circumstances, this cross-talk can be induced in the absence of a cell surface receptor for the virus, in which case, infection can proceed efficiently. This new evidence strongly implies that the role of cell surface receptors in infections of γ-retroviruses (and perhaps of other enveloped animal viruses) is more complex and interesting than was previously imagined.

In all these cases, the EnvGP have a double function attachment to the receptor and an exclusive role in fusion in the same protein. However, for other viruses, these two functions are filled by different proteins. The E2 protein of the

Table 4.1. Example of Different Viruses, Their Envelope Glycoproteins, and Their Receptors

Virus Family	Virus	Envelope Glycoprotein	Receptor	Function
pH-dependant				
Alpha	Semliki forest virus (SFV)	E3-E2-6K-E1	MHC-I	Immune recognition (1 tmd)
	Chikungunya	E3-E2-6K-E1		
Flavi	Tick-borne encephalitis virus (TBEV)	PrM-E	HS	Glycoaminoglycan (1 tmd)
	Dengue	PrM-E	MR (CD206)	Mannose receptor
			HS, DC-SIGN?	Glycoaminoglycan, lectin
Hepaci	Hepatitis C virus (HCV)	E1–E2	CD81,	Tetraspanin (adhesion, activation. . .) (4 tmd)
				HDL Receptor (2 tmd)
			SRB1,	Tight junction (4tmd)
			Claudin,	Tight junction (4tmd)
			Occludin	
Orthomyxo	Influenza A	HA1–HA2	Sialic acid	Carbohydrate
Rhabdo	Vesicular stomatitis virus (VSV)	G	PS?	Phosphatidylserine
Bunya	Haanta virus	G1–G2	beta 3 integrin	Integrin (1tmd)
			DAF(CD55)	Complement system
Filo	Ebolavirus	GP1–GP2	L-DC-SIGN,	C-type lectin (1tmd)
			hMGL	C-type lectin (1tmd)
			Cathepsine L, B	Endosomal protease
			FRα	Folate receptor α (1tmd)
Rubella	Rubellavirus	E2-E1	PS, PI, PE, PC	Phospholipids
			ganglioside	Glycolipids
Retro	Avian leukosis and sarcoma virus (ALSV)	Gp85–gp37	Tva	LDL-R homology (1tmd)
Arena	Lymphocytic choriomeningitis virus (L-CMV)	GP1–GP2	α-Dystroglycan	Laminin receptor extracellular matrix
	Lassa virus	GP1–GP2	α-Dystroglycan	Laminin receptor extracellular matrix

(*Continues*)

Table 4.1. (*Continued*)

Virus Family	Virus	Envelope Glycoprotein	Receptor	Function
pH-Independent				
Retro	Human immunodeficiency virus 1 (HIV-1)	Gp120–gp41	CD4,	Immune recognition (1 tmd)
			CCR5, CXCR4	Chimiokine receptor (7 tmd)
	Amphotropic Murine leukemia virus (A-MLV)	Gp70–p15	PiT-2	Na-Pi cotransporter (10 tmd)
	Feline leukemia virus (FeLV)	Gp70–p15	PiT-1	Na-Pi cotransporter (10 tmd)
	Gibbon ape leukemia virus (GaLV)	Gp70–p15	PiT-1	Na-Pi cotransporter (10 tmd)
	Pig endogenous retrovirus A (PERV-A)	Gp70–p15	HuPAR2, GHBh1	G-protein coupled receptor (10 tmd)
	Feline endogenous retrovirus (RD114)	GP70–p15	ASCT-2, SLC1A5	Cotransport Na-a.a. neutres (7 tmd)
	Human T-cell lymphotropic virus type 1 (HTLV-1)	Gp46–gp21	GluT-1	Transport glucose (12tmd)
			Neuropilin 1	VEGF receptor, synapse(1tmd)
			HS	Glycoaminoglycan (1 tmd)
Paramyxo	Measle virus	H, F	CD46	Complement regulator
			SLAM (CD150)	Ig-like, CD4 regulation
	Newcastle disease virus	HN, F	Sialic acid	On gangliosides and glycoproteins
	Human parainfluenza virus	H, F2-F1		
Alpha-herpes	Herpes simplex virus 1 (HSV-1)	gB, gD, gL, gH	HVEM,	Co-stimulation factor (1 tmd)
			nectin 1α et β,	Ig-like; adherens-junction, synapse (1 tmd)
			HS	Glycoaminoglycan (1 tmd)
Beta-herpes	Epstein-Barr virus (EBV)	gB, gL, gH	CD21	Complement cascade component
Pox	Vaccinia virus	L1, A27L, D8L, A33, H3L, B5, etc.	HS	Glycoaminoglycan (1 tmd)
			EGF receptor	Signaling receptor
Corona	Murine hepatitis virus 4 (MHV-4)	S	Bgp (biliary gp)	Ig-like (1 tmd)
	SARS-CoV	S	ACE-2	Metallocarboxypeptidase
			Cathepsine L, B	Endosomal protease

SFV alphavirus binds the receptor that permits the endocytose of the virus in a compartment where the acid pH will activate the E1 protein that will induce the fusion (Table 4.1). For most paramyxoviruses (including Newcastle parainfluenza virus, human parainfluenza virus type 3 (HPIV-3), mumps virus, bovine RSV (BRSV), ovine RSV (ORSV), and human RSV (HRSV)), the hypothesis is that the binding of HN or G/SH to their receptor induced not only conformational changes in the receptor but also in the F protein with which it interacts. These changes make F competent for the membrane fusion, with the exception of the F protein of the simian parainfluenza virus 5 (SV5), the BRSV, ORSV, and HRSV that do not strictly require HN or G/SH to induce the fusion (though the fusion is greatly increased by HN or G/SH, respectively). This complexity in the distribution of the functions is still more evident in, for example, the alphaherpesvirus (HSV-1, HSV-2) (Rey, 2006). In this case, the binding of the gD glycoprotein to one of its three receptors (HVEM for herpesvirus entry molecule mediator, or nectin 1, or a specific heparan sulfate) activates a complex of gB trimers associated to gL and gH proteins, all the three being essential to the fusion (Gianni *et al.*, 2006; Table 4.1). In the same way as for the hepatitis C virus, the E1E2 heterodimer induces entry following the recognition of at least four receptors, CD81, SRB1, claudin, and ocludine (Zeisel *et al.*, 2009).

Another interesting variation of activation of fusion is the artificial reverse process. In some cases, it was described that some exosomes or pseudoparticle-incorporating receptors were able to enter cells expressing envelope. For example, the multitransmembrane receptor mCAT-1 from ectropic murine leukemia virus (MLV) or the Tva receptor for avian sarcoma-leukosis virus (ASLV-A) can be used to infect cells that express their respective retroviral EnvGP (Balliet and Bates, 1998). Similarly, the incorporation of both CD4 and one other coreceptor of human immunodeficiency virus (HIV), CCR5 (Endres *et al.*, 1997) or CXCR4 (Endres *et al.*, 1997; Mebatsion *et al.*, 1997; Schnell *et al.*, 1997), allows the production of viral particles infecting cells that express simian immunodeficiency virus (SIV) or HIV envelope glycoprotein. Interestingly, it was shown that Nef, an accessory protein of human immunodeficiency virus type 1 (HIV-1) that enhances the infectivity of progeny virions when expressed in virus-producing cells, significantly enhanced the infectivity of CD4-chemokine receptor pseudotypes for cells expressing HIV-1 Env. Surprisingly, Nef also increased the infectivity of HIV-1 particles pseudotyped with Tva, even though Nef had no effect if the pH-dependent Env protein of ASLV-A was used for pseudotyping (Pizzato *et al.*, 2008). This process indicates that the difference of superficial tension between the cell (weak because the radius of the cell is big) and the virus (strong as the radius of the virus is small) is not crucial for the development of an oriented fusion process with EnvGP on the one side and receptor on the other. Having said that, the cell is not an empty bubble, and this efficacy of the fusion process regardless of which membrane harbors the receptor

or the EnvGP may reflect the adaptation of the virus to optimize the membrane merging independently of the superficial tension. Cells are not completely round, and superficial tension depends on the localization where the virus binds. Moreover, the plasma membrane has different lipid compositions and interacts with the cytoskeleton which will attenuate or increase the superficial tension at certain localizations.

D. Structural classification: Class I, class II, and class III fusion proteins

Glycoproteins from enveloped viruses have evolved to combine two main features. First, they have the capacity to bind with a specific cellular receptor and second, they include a fusion domain (peptide fusion and transmembrane domain) that can be activated to mediate the merging (fusion) of viral and cellular membranes.

Three different classes of viral fusion proteins have been identified to date based on key structural features at pre- and postfusion stages. Many studies have demonstrated that the structural transition from a pre- to a postfusion conformation leads to a stable hairpin conformation. This includes class I fusion proteins, characterized by trimers of hairpins containing a central alpha-helical coiled-coil structure, and class II fusion proteins, characterized by trimers of hairpins composed of beta structures. A third class of fusion proteins has been described recently, that also forms trimers of hairpins by combining the two structural elements alpha-helix and beta-sheet structures (Roche *et al.*, 2006, 2007).

The synthesis and the conformation of these classes I, II, or III fusion proteins are different. Class I viral fusion proteins of diverse virus families, including Retroviridae, Filoviridae, Orthomyxoviridae, Paramyxoviridae, and Coronaviridae, differ greatly in size and amino acid sequence, but their membrane-anchored domains share common structural features that are essential for membrane fusion, including two heptad repeats (called HR-1 and HR-2), preceded by a hydrophobic fusion peptide. Class I membrane-fusion reaction is mediated by the refolding of the fusion protein to a highly stable rod-like structure with a central trimeric α-helical coiled coil. Such coiled-coil structures are emblematic of class I proteins, and physical demonstration or computer prediction of such a structure is frequently used to help define a fusion protein as belonging to class I. The envelope glycoprotein of a retrovirus is generated by the cleavage of its precursor in one SU and one transmembrane subunit containing an anchoring sequence to the membrane (Hunter, 1997). This maturation, essential to the process of fusion, frees a hydrophobic sequence to the N terminal extremity of the TM, named fusion peptide, as it is supposed to insert into the target membrane and initiates the fusion. Initially, the fusion peptide is masked inside the trimer of EnvGP, a form competent for the fusion (Fig. 4.2A).

In general, the cleavage of the fusion proteins is mandatory for most class I proteins to render them competent for fusion (Earp *et al.*, 2005; Harrison, 2005). Exceptions are the envelope glycoprotein from Ebola or SARS-CoV viruses, classified as class I on the grounds of the three-dimensional structure of one of their fragments, which are not cleaved but yet are functional. Though an N-terminal fusion peptide is predicted in the potential transmembrane subunit (localized by analogy to homologous viruses), their functionality can be explained also by the presence of an internal fusion peptide (Ebola). Another explanation of their functionality is their requirement for the L or B cathepsins that cleave the EnvGP during endocytosis (see below). The number of complexes on the surface of viruses harboring class I fusion proteins is very variable. It seems that lentiviruses have only 10–20 SU–TM trimers, whereas the coronaviruses have hundreds of trimers coating the surface, giving them their characteristic morphology of "crown" which is the origin of the name of this family (Tables 4.1 and 4.2).

The process of assemblage and biosynthesis of class virus II is very different to the class I proteins (Kielian and Rey, 2006) (Fig. 4.2B). During biosynthesis, the alphavirus (E1) and flavivirus (E) fusion proteins fold cotranslationally with a companion or regulatory protein, termed p62 (or PE2) for alphaviruses and prM for flaviviruses (Garoff *et al.*, 2004). This heterodimeric interaction is important for the correct folding and transport of the fusion protein. Both p62 and prM are cleaved by the cellular protease furin late in the secretory pathway, in a maturation reaction that is a crucial regulatory step for subsequent virus fusion (Salminen *et al.*, 1992; Stadler *et al.*, 1997; Wengler, 1989; Zhang *et al.*, 2003a). Though this cleavage in the envelope glycoprotein complex is important, contrary to class I fusion protein, it is not crucial, since mutated alphavirus and flavivirus for which the regulating proteins are not cleaved have only a decreased infectivity (Salminen *et al.*, 1992; Zhang *et al.*, 2003b). In the case of the SFV alphavirus, the fusion process induced by the uncleaved fusion proteins can be a trigger after treatment of the virus at pH5 or less, rather than the normal fusion threshold pH 6 of the wild-type virus (Salminen *et al.*, 1992; Zhang *et al.*, 2003a). This variation in the threshold of pH of the fusion is necessary for the dissociation of the heterodimer. The class II fusion proteins are either homodimers or heterodimers (E homodimer for the flaviviruses or E2–E1 heterodimer for alphaviruses) that form an envelope netting the viral membrane (Lescar *et al.*, 2001; Rey *et al.*, 1995). The internal fusion peptide is masked at the interface of the dimers at the extremity of a long beta sheet. One important difference between these two groups of viruses is the budding site (Garoff *et al.*, 2004). In alphaviruses, the p62–E1 complex is transported to the plasma membrane, and the heterodimer interaction is maintained after p62 processing. New virions bud at the plasma membrane, in a process that is driven by lateral contacts between E2 and E1 heterodimers (E2 being the mature companion protein) to induce the required curvature of the lipid bilayer, in combination

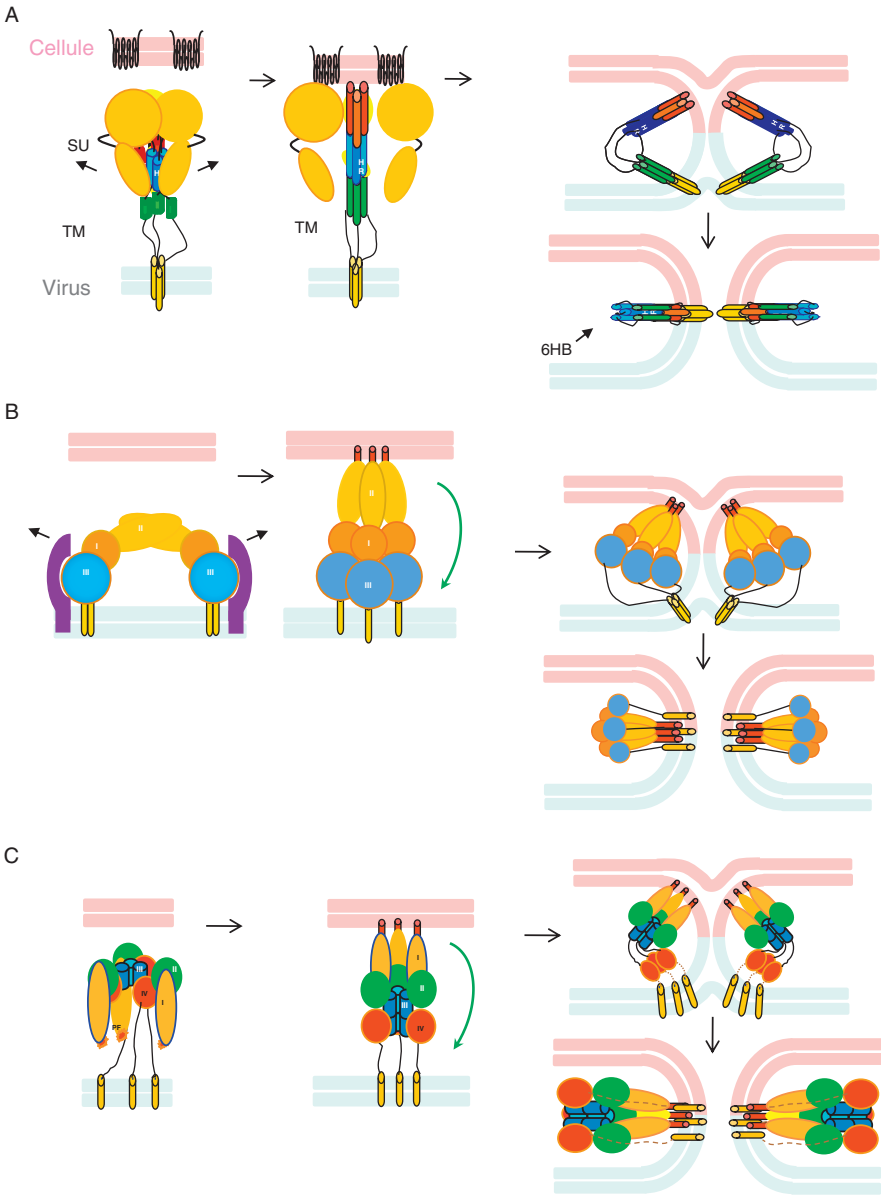

Figure 4.2. Possible fusion models have evolved for class I (A), class II (B), and class III (C) fusion proteins from their pre- to postfusion conformations. Ambiguities remain regarding certain events in membrane fusion promoted by a viral fusion protein. Despite the diversity in the structure of the fusion proteins, the major steps of the fusion process are similar. The first step, after EnvGP activation upon receptor binding or acidification

with interactions of the cytosolic tail of E2 with the nucleocapsid. Budding results in the formation of icosahedral-enveloped particles of triangulation $T=4$, containing 80 trimeric E2/E1 spikes.

By contrast, flavivirus particles bud into the endoplasmic reticulum as immature virions formed by 60 trimers of prM–E. The immature particles have an organization similar to that of mature alphaviruses, with each trimer forming a

of the endosomal compartment, exposes the fusion peptide that is projected toward the top of the glycoprotein, allowing the initial interaction with the target membrane. The second step is the folding back of the C-terminal region onto a trimeric N-terminal region that leads to the formation of a postfusion protein structure. The third and final step also requires further refolding of the membrane proximal and transmembrane regions in order to obtain a full-length postfusion structure where both membrane anchors (fusion peptide and tm domains) are present in the same membrane. Three different classes of fusion have been identified so far based on common structural motives. (A) The class I fusion proteins are characterized by trimers of hairpins containing a central alpha-helical coiled-coil structure. For retroviruses, receptor binding induces the movement of the SU, allowing a loop-to-helix transition of a polypeptide segment of TM that was previously buried underneath the SU heads, projecting the fusion peptide ~100 Å toward the target membrane, where it inserts irreversibly. This occurs by a "spring-loaded" mechanism. The HR2 C-terminal end (green) of the long TM α-helix jackknifes back, reversing the direction of the viral-membrane-proximal segment of TM, which then interacts in an antiparallel fashion with the groove formed by the N-terminal HR1 (blue) trimeric coiled coil. The final postfusion conformation of TM is, therefore, a highly stable rod with the TM and fusion-peptide segments together at the same end of the molecule, a structure termed a "trimer of hairpins" or helix buddle (HB). (B) Class II fusion proteins are characterized by trimers of hairpins composed of beta structures. The red, yellow, and blue parts of each subunit correspond, respectively, to domains I, II, and III of the ectodomain. The fusion loop is at the tip of domain II. Monomeric transition between the prefusion dimer and the trimeric-extended intermediate is shown. After exposure to the low pH of the endosomes, domains I and II swing outward, while domain III and the stem remain oriented against the membrane roughly similar to the prefusion state. The fusion loop, at the top of the diagram, interacts with the target bilayer. Domains I and II associate into the trimeric core of the postfusion conformation, and domain III must then zip back along the trimer core, thus reorientating the domain III. (C) A third class of fusion proteins has been described recently, which also forms trimers of hairpins by combining the two structural elements alpha-helix and beta-sheet structures. Class III fusion proteins are composed of five domains that give rise to a molecular architecture very distinct from any reported class I or class II fusion proteins. Interestingly, the ectodomain of G has been crystallized in its pre and postfusion (low-pH) state. During the conformational change that occurs upon low pH exposure, the domains of G radically change their position and orientation as a result of rearrangements that occur in the linker regions. Domain I (yellow), carrying the fusion loops, and the transmembrane domain move 16 nm from one end of the molecule to the opposite (Backovic and Jardetzky, 2009). Only domain III (blue) undergoes significant refolding with extension of the central helix F. To complete the process, the C-terminal helices of domain IV (red) insert into crevices formed by two other protomers in the postfusion form, reminiscent of the structural changes observed during refolding events of class I fusion proteins with HB formation.

Table 4.2. Classification of Fusion Proteins Based on Their Family, Class, and Activation Mechanism

Virus family	Virus species	Fusion proteins	Fusion pH for activation
Class I			
Orthomyxoviridae	Influenza A virus	HA2	Low
	Influenza C virus	HEF	Low
Paramyxoviridae	Simian parainfluenza virus 5	F (F2–F1)	Neutral
	Human parainfluenza virus	F	Neutral
	Newcastle disease virus	F	Neutral
	Respiratory syncytial	F	Neutral
Filoviridae	Ebola virus	Gp1-Gp2	Low (for cathepsin cleavage)
Retroviridae	Moloney Murine leukemia virus	TM (gp21)	Neutral
	Human immunodeficiency virus 1	gp41	Neutral
	Simian immunodeficiency virus	gp41	Neutral
	Human T cell leukemia virus 1	gp21	Neutral
	Human syncytin-2	TM	Neutral
	Visna virus	TM	Neutral
Coronaviridae	Mouse hepatitis virus	S2	Low (for cathepsin cleavage)
	Sars corona virus	E2	Low (for cathepsin cleavage)
Class II			
Flaviviridae	Tick-borne encephalitis virus	E	Low
	Dengue virus	E	Low
Togaviridae	Semliki forest virus	E1	Low
Class III			
Rhabdoviridae	Vesicular stomatitis virus	G	Low
Herpesviridae	Herpes simplex virus	gB	Neutral
	Eptsein Barr virus	gB	Neutral

There is no correlation between these criteria for class I and class III fusion proteins, but all the viruses harboring class II fusion proteins are pH-dependent.

spike in which prM covers the fusion protein E. The newly formed virions are then transported to the external milieu through the exocytic pathway. Processing of prM generates the mature M protein with a short (~40 residues) ectodomain (Kuhn *et al.*, 2002; Zhang *et al.*, 2003b). Presumably because of the removal of a large portion of the prM ectodomain, the flavivirus surface dramatically reorganizes after processing to give 90 E–E homodimers arranged with icosahedral symmetry. The mature flavivirus particles display a smooth, spikeless surface, with E dimers ordered in a characteristic "herring bone" pattern.

Finally, the four class III fusion proteins resolved (VSV-G, gB of HSV1 and EBV and baculovirus gp64) in spite of their homology of trimeric structure have some major functional differences arising from their difference of biosynthesis. G protein is the only class III fusion protein whose prefusion structure is known. The proteins of class fusion III have a combination of the two structural elements, alpha helix like class I and beta sheet like class II (Backovic *et al.*, 2009; Heldwein *et al.*, 2006; Kadlec *et al.*, 2008; Roche *et al.*, 2006, 2007) (Fig. 4.2C). The trimers are maintained by interaction between central alpha helixes, but each domain of fusion exposes two buckles of internal fusion peptide placed at the extremity of a long beta sheet. The G protein is the only protein responsible for the binding and the entry of the VSV. The rhabdovirus VSV possesses 1200 molecules of the G protein on its surface and forms 400 trimers. In contrast, the HSV-1 virus incorporates 12 different EnvGP on its surface, of which 4 are essential for the entry step (gD, gB, and gH/gL) (Turner *et al.*, 1998). During their biosynthesis, neither the G protein of VSV nor gB of HSV-1 are cleaved, and they present internal fusion peptides. However, whereas the G is functional by itself, the gB envelope glycoprotein alone is not sufficient to induce the entry of the virus or the membrane fusion. Nevertheless, currently, no precise interaction between gD, gB, and gH/gL has been identified, even though the gD ectodomain has been shown to allow the entry of engineered HSV-1 virus particles that lack gD (that is gD-null mutants; Cocchi *et al.*, 2004). Gp64 is the major component of the viral envelope, and the sole fusogenic proteins that are triggered to induce the fusion in the low pH environment of endosomes for baculovirus. Interestingly, the distinguishing feature between G and gp64 and any other fusion protein is that they can undergo a reversible conformational change, unlike class I and most class II fusion proteins, for which the postfusion conformation is thermodynamically more stable at all pH values, and the conformational rearrangement is effectively irreversible. VSV or gp64 exposure to low pH inactivates the virus, but the fusion activity can be fully recovered when the pH is raised. It has been proposed that the reversibility of the conformational change allows G to avoid unspecific activation during transport through the acidic Golgi vesicles.

As seen in the previous sections, though there is much described variation in the manner of activation of the fusion proteins and additional mechanisms await discovery, only three classes of fusion proteins have been defined so far (Weissenhorn *et al.*, 2007). As previously described, based on the main structural organization of their EnvGP, the viruses are now assigned to the class I, II, or III. In parallel, based on the activation mechanism of the fusion protein, viruses have been classified as pH-dependent or -independent. Interestingly, the relationship between the mechanism of activation of the fusion and the class of protein of fusion is not correlated. The HIV or Influenza EnvGP are both class I and yet they have a process of activation that is pH-independent and

-dependent, respectively, for the entry of the virus. Similarly, the class III fusion proteins enclose the Herpes virus simplex 1 (HSV-1) gB protein and the rhabdovirus VSV-G protein that are pH-independent and pH-dependent, respectively, for their activation. So far, all the class II fusion proteins have been pH-dependent for their activation.

E. Common refolding process

Although there are notable differences between the activation processes, the structural motives used, and the initial oligomeric states of the fusion proteins (the native trimeric conformation of class I and III proteins in opposition to the homo- or heterodimers of the class II fusion proteins), the common features of the final structures obtained after fusion seem to suggest some generic mechanism of conformational changes common to all EnvGP of enveloped viruses (see Fig. 4.2).

First, the activation of the fusion protein following the interaction with the cellular receptor(s), coupled or not to the exposure to the acid environment of the endosomes, exposes the fusion peptide that is projected toward the top of the glycoprotein, allowing the initial interaction with the cellular target membrane. For class I fusion proteins, the proposed model indicates that the transition of conformation requires the transformation of a part of the molecule in alpha helix and the association of this in three helixes bundle (named the "coiled coil"; Weissenhorn *et al.*, 2007). For retroviruses, this movement allows a loop-to-helix transition of a polypeptide segment of TM that was previously buried underneath the SU heads, projecting the fusion peptide $\sim$100 Å toward the target membrane, where it inserts irreversibly. In the case of class I fusion proteins like retroviruses, this occurs by a "spring-loaded" mechanism. This initial change is proposed to result in a "prehairpin intermediate," an extended structure that is anchored both in the target membrane by the fusion peptide and in the virus membrane by the TM transmembrane segment. For the class II fusion proteins, the projection of the fusion peptide requires the dissociation of the hetero- or homodimers and modifications in the "hinge region" unstructured before conformational changes (Stiasny and Heinz, 2006). For the class III (Roche *et al.*, 2008), similarly to the fusion proteins of class I, the exhibition of the fusion peptide requires a rearrangement of the domains mediated by modification of the central helixes that do remain parallels.

Second, the folding back of the region including the fusion peptide onto a trimeric C-terminal region leads to the formation of a postfusion protein structure with the outer regions zipped up against an inner trimeric core. Interestingly, it has been described that all the class I, II, and III peptides can inhibit this step by competing with the interaction of EnvGP-specific domain for the formation of this structure.

Third, the final steps require further refolding of the juxtamembrane and transmembrane regions to obtain a stable postfusion structure with the fusion peptides and the transmembrane domains at the same extremity of a stable stem of a protein complex anchored in the target membrane. This structure brings the two membranes proximal and provides free energy to overcome the barrier of membrane merging (Melikyan, 2008). Membrane fusion occurs, which leads to pore formation and release of the viral genome into the cytoplasm.

The refolding of class II fusion proteins generates trimers from monomeric intermediate. The existence of monomeric intermediates for class I and III is not well known. However, if the steps that led to exposition of the fusion peptide and its interaction with the membrane targets maintain a three-order symmetry, the refolding of the C-terminal region requires the destruction of the trimer at least to the juxtamembrane region. Moreover, from the differences observed between the amino acids involved in the interface of the reconstituted resolved trimers in their pre- and postfusion conformations, it is probable that the conformational changes are going through a monomeric intermediate for the class III G envelope glycoprotein of VSV and the class I F protein of paramyxovirus. On the contrary, the interface of the class I HA2 subunit of the HA envelope glycoprotein trimer is very similar between the pre- and postfusion conformation, shedding doubt on the existence of monomeric intermediates. In contrast, when fusion is initiated at low pH, the dimers of the class II fusion proteins are broken, freeing monomers that reassociate in trimers.

Finally, precise structural information of the native metastable conformation (prefusion) and the final stable conformation (postfusion) is available only for a limited number of viruses (for envelope glycoprotein of influenza virus, SFV, TBEV, VSV, and the parainfluenza 5 F protein). The structural conversion of the native metastable conformation in a final stable conformation is not precisely known and is highly speculative for most viruses, and the envelope glycoprotein domains implied in these molecular rearrangements are little-referenced. Clearly, more studies are necessary to identify the intermediaries of envelope glycoprotein conformations. These intermediates would identify the domains that interact during the conformational changes which will highlight ways to generate inhibitory peptides.

F. Domain organizations/fusion domains

1. Acquisition of fusion competence: Priming by cleavage

During virus production, the host cell is basically preserved, since the expression of fusogenic competent glycoproteins is highly controlled for most viruses. However, for some viruses, the EnvGP induce a cytopathic effect that leads to

the generation of multinucleated cells, called syncytia, which are induced by the fusion of cells that express the envelope glycoprotein and receptors. Abundant glycoprotein at the surface of the cell could induce cellular death by syncytia formation, toxicity via receptor interaction, or immune recognition. For these reasons, the localization and the amount of the oligomerized envelope glycoprotein at the host cellular surface are highly modulated by fine trafficking and sequestration mechanisms. The receptor interference mechanism (either by saturation of binding site on receptor by envelope glycoprotein or by internalization of receptor by different viral proteins, as it is achieved by some retroviruses; Hunter, 1997) can also limit the amount of receptors available for fusion between infected cells. The control of the cleavage of the EnvGP to free the fusion peptide is also a regulation process of the fusion protein fusogenicity (Labonte and Seidah, 2008). Finally, EnvGP fusion competency may be a late event that occurs during virus budding, as described for MuLV retroviruses (Rein *et al.*, 1994).

Proteolytic priming is a common method of controlling the activation of membrane fusion mediated by viral glycoproteins. The members of the proprotein convertase (PC) family play a central role in the processing and/or activation of various protein precursors involved in many physiological processes and various pathologies such as neurodegenerative pathology, cancer bacterial toxins activation, and viral infections. The proteolysis of these precursors that occurs at basic residues within the general motif (K/R)-(X)-(K/R) is mediated by the proprotein convertases PC1/3, PC2, Furin, PACE4, PC4, PC5 (also called PC6), and PC7 (also called PC8, LPC, or SPC7), whereas the proteolysis of precursors within hydrophobic residues performed by the convertase S1P/SKI-1 and the convertase NARC-1/PCSK9 seems to prefer to cleave at a LVFAQSIP motif (Lahlil *et al.*, 2009). The seven PCs have different, albeit partly, overlapping expression patterns and subcellular localization. They have conserved aminotermini with highest homology in the subtilisin-like catalytic domain. Data on various infectious viruses revealed that the cleavage of their envelope glycoprotein precursors by one or more PCs is a required step for the acquisition of the infectious capacity of viral particles. Indeed, various studies have demonstrated the capacity of the PCs to correctly cleave a variety of viral surface glycoproteins. These include the HIV-1 gp160 (Decroly *et al.*, 1996) and surface glycoproteins of Hong Kong, Ebola virus, the severe acute respiratory syndrome coronavirus and chikungunya virus (Basak *et al.*, 2001; Bergeron *et al.*, 2005; Ozden *et al.*, 2008). In parallel, other studies revealed that the inhibition of processing of these viral surface glycoproteins by the PC inhibitors such as dec-R-V-K-R-CMK completely abrogated the virus-induced cellular cytopathicity. The surface glycoproteins of other viruses, particularly the hemorrhagic fever viruses (Arenaviridae family), such as Lassa (Basak *et al.*, 2002; Lenz *et al.*, 2001), Crimean Congo hemorrhagic fever (Vincent *et al.*, 2003), and lymphocytic choriomeningitis (Beyer *et al.*, 2003), were shown to be cleaved by the

convertase SKI-1 that cleaves at hydrophobic residues. Similarly, blocking of SKI-1 activity by a specific inhibitor has also shown to affect the processing and the stability of the glycoproteins of these viruses (Pullikotil *et al.*, 2004).

For all highly pathogenic avian influenza (HPAI) viruses of subtypes H5 and H7 known to date, the cleavage of HA occurs at the C-terminal R residue in the consensus multibasic motifs, such as R-X-K/R-R with R at position P4 and K-K/R-K/T-R with K at P4, and leads to systemic infection. Early studies demonstrated that the ubiquitously expressed furin and proprotein convertases (PCs 5 and 6) are activating proteases for HPAI viruses (Basak *et al.*, 2001; Stieneke-Grober *et al.*, 1992). Recently, ubiquitous type II transmembrane serine proteases, MSPL and its splice variant TMPRSS13, have been proposed as novel candidates for proteases processing HA proteins of HPAI (Okumura *et al.*, 2010).

Is it interesting to note that viral receptors can also be modified by proprotein convertase. Indeed, PCSK9 impedes hepatitis C virus infection *in vitro*, modulates liver CD81 expression, and enhances the degradation of the low-density lipoprotein receptor (LDLR) (Labonte *et al.*, 2009), bestowing on the proprotein convertase an additional role in controlling the fusogenicity of the envelope glycoprotein.

2. Fusion peptide

The exhibition and insertion of a hydrophobic fragment of 10–30 residues in the membrane, named "fusion peptide" or "fusion loop" is a crucial step of the fusion process (Epand, 2003). The fusion peptides in an N-terminal position (such as for the retrovirus or the influenza virus) is liberated for most viruses after envelope glycoprotein cleavage, and it can insert into the external layer of the membrane in an oblique manner, whereas the fusion loop (for the class II and III viruses) remains probably more superficial (see Table 4.3). The fusion peptide of the class I and II proteins is initially buried in the envelope glycoprotein trimer or dimer, respectively. For the class III, the fusion loop is present outside of the structure, most likely because the fusion peptide of class III fusion proteins is weakly hydrophobic and probably requires a cooperation between several loops to be functional and efficient. The simple picture of a viral fusion protein acting on cell and viral membranes by means of only two restricted segments, that is to say, the fusion peptide and the transmembrane domain, is too simplistic. Instead, a more complex concerted action of different membranotropic segments of the fusion proteins is necessary. More conformational changes are required to achieve a complete fusion of the two lipid bilayers. As described previously, the class I–III fusion proteins roughly share common refolding processes and formation of intermediates. Several regions of the fusion protein complex indirectly aid the fusion process, as for example, the "stem" regions (see below).

Table 4.3. Fusion Peptide Characteristics from the Fusion Protein from Different Classes

Fusion peptide	Class I	Class II	Class III
Initial situation	Buried in trimer interface	Buried in the dimer interface	Buried in the interface between different trimers
Localization	N-term (HIV, HA2, etc.) or internal (RSV, Ebola, etc.)	Internal loop embedded between 2 beta-strands	2 internal loop (segmented fusion peptide; nonobvious on primary sequence)
Structure flexibility	Alpha helix ↔ random coil/turn	Stable random ↔ coil and turn	Alpha helix ↔ random coil/turn
Interaction with membrane	Insert into one bilayer leaflet	Stay at the membrane surface (insert into hydrocarbon chains of the outer leaflet)	Stay at the membrane surface (insert into hydrocarbon chains of the outer leaflet)
Maturation to prefusion state through	Proteolytic processing of fusion protein (except Ebola, Sars)	Proteolytic processing of companion protein	No proteolytic process
Activated in the glycoprotein complex	2 proteins (PIV 5) 1 cleaved protein (HA) 1 uncleaved (Ebo,SARS)	2 identical or different proteins (SFV, TBEV)	1 uncleaved protein (G) 3 proteins (gB with gH/gL)

Contrary to the relatively simple and canonical organization of the fusion peptides for the influenza virus or the flavivirus E protein, the recently resolved structures of the gB glycoprotein of the simplex herpes type 1 virus (Heldwein *et al.*, 2006) and G protein (Roche *et al.*, 2006, 2007) of the vesicular stomatitis virus (VSV) indicated a bipartite fusion peptide composed of two hydrophobic loops, each loop being relatively nonpolar or very weakly hydrophobic (which rarely leads to the identification of a fusion peptide by fusion peptide prediction based on hydrophobic domain identification). These differences in the organization of the fusion peptides suggest differences of action of the fusion proteins, notably in the number required. According to experiments using neutralization assays, it seems that one HIV envelope glycoprotein is capable of inducing membrane fusion. However, it has been shown that the induced fusion by HA requires a collaboration of several complexes of envelope glycoprotein (8–9 trimers). In the same way, different networks of class II fusion proteins have been proposed, such as the hexagonal organization observed on the surface of liposomes or an association of five trimers in a structure similar to a volcano based on structure predictions (Stiasny and Heinz, 2006). Concerning the fusion protein of class III, a hexagonal structure has been proposed for rabies virus envelope glycoprotein and recently for VSV-G according to a modeling using its structure. The requirement for multiple fusion peptides may be compared to the

number of receptors required. The assembly of a complex containing several receptors may be a prerequisite for the membrane fusion steps that require multiple EnvGP molecules to cooperatively participate in this process. For example, in the case of HIV-1, the presence of more than one CD4 in contact with the virus enhances the infectivity dramatically and reduces the concentration of coreceptors needed for infection (Platt *et al.*, 1998). Further investigation of this system has implied that a critical complex containing approximately four to six coreceptors is a requirement for infection, although it is not known whether this complex performs a transient role and then disperses or is maintained throughout the membrane fusion process (Kuhmann *et al.*, 2000). Despite some uncertainties, several lines of evidence have suggested that three to six hemagglutin trimers may cooperatively participate in the influenza A virus-mediated membrane fusion reaction (Blumenthal *et al.*, 1996; Boulay *et al.*, 1988) and that multiple envelope glycoprotein trimers are required for rabies virus-mediated membrane fusion (Roche and Gaudin, 2002). see Table 4.3.

3. The role of cytoplasmic tail in fusion and influence of its length

The cytoplasmic tails of envelope viruses harbor different motifs that are responsible for its trafficking and are variable in length. It is surprising to see that the cytoplasmic tails of fusion proteins are not exchangeable. For example, when the HCV E1 and E2 cytoplasmic tails or the F cytoplasmic tails of HRSV (Human Respiratory Syncytial virus) are substituted for that of VSV-G envelope glycoprotein (or CD4), the fusogenicity of these envelopes in cell–cell fusion assays and virus–cell assay (infection) is destroyed (Buonocore *et al.*, 2002; Oomens *et al.*, 2006). Similarly, when the HA glycoprotein is anchored by a GPI, the entry process is stopped at the hemifusion step (Kemble *et al.*, 1994; Markosyan *et al.*, 2000). The cellular localizations (e.g., cholesterol-rich microdomains) are sometimes modified, and biochemical modifications (glycosylation, oligomerization) can affect the properties of the VSV-G EnvGP (Kemble *et al.*, 1994). Nevertheless, in their initial context, these cytoplasmic tails allow fusion.

For some viruses, regulation of fusion is mediated by the cleavage of the cytoplasmic tail. For γ-retrovirus, in addition to the SU–TM cleavage, a significant fraction of virion-associated TM is further processed by the viral protease removing the C-terminal 16 amino acids of the cytoplasmic domain, the R peptide. γ-Retrovirus virions assemble and bud from infected cells as immature particles that must undergo an additional proteolytic maturation to become infectious (Green *et al.*, 1981; Lavillette *et al.*, 1998, 2002; Rein *et al.*, 1994). This maturation concerns the viral protease-dependent cleavage of the so-called peptide R at the C-terminus of the cytoplasmic tail. The R peptide inhibits fusion, and different hypothesis have been proposed. First, the R peptide contains

an Y-X-X-internalization motif, and the removal of this motif following the cleavage of the R peptide might result in a higher amount of envelope at the surface membrane and thus more fusion (Song *et al.*, 2003). Second, following the R peptide cleavage, the remaining cyt tail forms a membrane-embedded amphiphilic alpha-helix domain that destabilizes the membrane (Rozenberg-Adler *et al.*, 2008; Zhao *et al.*, 1998). Third, it has been proposed that as the R peptide contains a palmitoylation, its removal induces the close trimerization of the cyt tail and drastic conformational changes in the ectodomain of Env (Aguilar *et al.*, 2003) which might influence Env fusogeneity by destabilizing SU–TM complexes. These conformational changes are necessary for the isomerization of the SU–TM disulfide MLV Env (Loving *et al.*, 2008). This R peptide cleavage is the last step leading to a fusion competent infectious MLV retrovirus, but this final modification does not exist in lentiviruses which harbor a long cytoplasmic tail. However, truncations of the long cytoplasmic domains of lentiviral Env proteins occur under certain culture conditions (Chakrabarti *et al.*, 1989) and increase Env fusogenicity in a similar way to mutated truncated versions of SIV, HIV-1, and HIV-2 Envs (Mulligan *et al.*, 1992; Spies *et al.*, 1994; Wilk *et al.*, 1992). This regulation is a hallmark of adaptation of endogenous retroviruses, as this cleavage is fulfilled by a cellular protease that activates the endogenous EnvGP HERV-W in relevant tissues involved in placenta development. It is interesting to note that for most viruses (and not only the γ-retrovirus), the truncation of the cytoplasmic tail increases the fusogenicity of the EnvGP, as is seen in paramyxoviruses (Moll *et al.*, 2002). In many cases, this truncation seems to increase the cell surface expression (since cellular trafficking signals in the cytoplasmic tails are modified), which may explain the increase of fusogenicity. For certain other Env, the truncation leads to conformational changes in the ectodomain which lowers the activation threshold for fusion, resulting in enhanced Env fusion activity and kinetics (Aguilar *et al.*, 2003; Cote *et al.*, 2008; Spies *et al.*, 1994; Zhao *et al.*, 1998). Finally, the cleavage of the cytoplasmic tail sometimes allows, at least partially, a cell–cell fusion at neutral pH (pH-independent), although the entry of the virus remains pH-dependent. The notion of pH-dependence, seems in this case, to be due to a specific conformation or a particular density of envelope glycoprotein (Cote *et al.*, 2008).

4. The transmembrane proximal region

Numerous studies on several viruses have highlighted the critical role of the pretransmembrane sequence (PTM), also called the membrane proximale region or the juxtamembrane domain (JMD), which is rich in aromatic amino acids. The class I fusion glycoproteins of coronavirus, lentivirus (HIV, SIV, FIV), ebola virus (Munoz-Barroso *et al.*, 1999; Saez-Cirion *et al.*, 2003), and many other

viruses contain this short JMD region in the ectodomain between the end of HR-2 and the beginning of the transmembrane (TM) domain (Salzwedel *et al.*, 1999). The class II (SFV, dengue, TEBV) and class III (VSV and the herpes virus) fusion proteins also possess these regions, although they are less rich in tryptophan than the class I JMD (Jeetendra *et al.*, 2002, 2003; Roche *et al.*, 2008). Although the JMDs of fusion proteins of enveloped viruses are rich in aromatic amino acids, the number, spacing, and sequence of the aromatic amino acids are quite variable; however, the function remains the same. These JMDs contribute to the conformational changes that occur during membrane fusion, interact with membranes, induce membrane destabilization, and/or facilitate membrane fusion (Munoz-Barroso *et al.*, 1999). Therefore, the JMD of viral fusion proteins is a potential target for viral inhibitors. Entry inhibitors that target the JMD of class I fusion proteins include monoclonal antibodies that bind the JMD of gp41 to the FIV and to the HIV (Lorizate *et al.*, 2006; Purtscher *et al.*, 1994). Some peptides that mimic the JMD have been designed for EnvGP of FIV (Giannecchini *et al.*, 2004), HIV (Moreno *et al.*, 2006), Ebola virus (Saez-Cirion *et al.*, 2003), and the SARS virus (Howard *et al.*, 2008) and inhibit viral entry. This strategy has been also broadly used against many other paramyxoviruses such as the Sendai virus (Joshi *et al.*, 1998; Rapaport *et al.*, 1995), the Newcastle disease virus (Young *et al.*, 1999), the human parainfluenza type 3 (HPIV-3) (Yao and Compans, 1996), the respiratory syncytial virus (RSV), and the measles virus (MV) (Lambert *et al.*, 1996). In the same way, peptides that mimic the JMD from class II and III fusion proteins have also been developed against infection by Dengue virus (Hrobowski *et al.*, 2005) and CMV (English *et al.*, 2006; Lopper and Compton, 2004). In the case of HCV, some juxtamembrane domains have been proposed in E1 and E2 (Drummer and Poumbourios, 2004; Drummer *et al.*, 2007). High-throughput screening (HTS) of peptides derived from E1 and E2 sequences has identified inhibitory peptides close to the transmembrane domain of E2, though they are not among the most inhibitory (Cheng *et al.*, 2008). However, these strategies suffer from certain limitations. The derived peptides of these JMD often have the capacity to oligomerize, which inactivates them, and some stratagems need to be implemented to make the peptides more bioreactive against viruses. Moreover, these peptides that prevent the correct conformational change of the envelope glycoprotein, must act in a certain window of time and in a particular compartment compatible with their active structure. Indeed, the acid pH of some compartment is not compatible with the bioactive structure of the peptide. For the SARS-CoV virus, the peptide is able to inhibit the entry of the virus in the presence of protease at the cellular surface, but the peptide has little effect on the entry of the virus by endocytosis or on the activation by cathepsin L in the acid conditions of the endosome (Ujike *et al.*, 2008). In the case of Influenza, it has not been possible to develop such inhibitory peptides targeting the juxtamembrane domain. Reasons suggested for this include the

entry through acid compartments which modify protein structures, the entry by different pathways of endocytose, and the rapidity of the conformation changes of HA. Some modifications can be made to these peptides to increase their efficiency for viruses that fuse at the plasma membrane. For three paramyxoviruses, HPIV-3, a major cause of lower respiratory tract diseases in infants, and the emerging zoonotic viruses Hendra virus (HeV) and NiV, which cause lethal central nervous system (CNS) diseases, the addition of cholesterol to a paramyxovirus HRC-derived peptide (derived from the heptad repeat immediately preceding the transmembrane domain) increased antiviral potency by 2 log units (Porotto *et al.*, 2010). This enhanced activity is the result of the targeting of the peptide to the plasma membrane. The cholesterol-tagged peptides on the cell surface create a protective antiviral shield, target the F protein directly at its site of action, and expand the potential utility of inhibitory peptides for paramyxoviruses.

III. HOW VIRUSES SUBVERT DIFFERENT CELL PROTEINS FOR ENTRY?

A. Definition of receptors, adsorption molecules, and cofactors

The definition of a receptor is very complicated and has limitations. It is sometimes difficult to distinguish between "simple" receptors that mediate adsorption or binding and that may not even initiate conformational changes, and "critical" receptors for fusion which, upon binding, will generate the conformational changes that will allow the exposure of the fusion peptide and lead to membrane fusion. Some cellular molecules are also involved in the localization or trafficking of viral receptors and these are important cofactors of entry. Another ambiguity is the role of enzymatic activities. Some are necessary to process the fusion protein inside producer cells, or inside the endosomes of target cells, and they are necessary to activate the potential of the EnvGP for membrane fusion. While it is inaccurate to consider them as "receptors", they are certainly critical cofactors.

1. Virus adsorption

All viruses likely bind at least weakly to multiple cell surface components such as heparan sulfate proteoglycans, DC-SIGN, integrins, or glycolipids (Bounou *et al.*, 2002; Cantin *et al.*, 1997; Fortin *et al.*, 1997; Jinno-Oue *et al.*, 2001; Mondor *et al.*, 1998; Saphire *et al.*, 1999, 2001). Although such binding substances probably do not induce conformational changes in EnvGP that are necessary for membrane fusion, they can enhance viral adsorption and substantially increase efficiencies

of infections, thus contributing to pathogenesis (Alvarez *et al.*, 2002; Bounou *et al.*, 2002; Geijtenbeek *et al.*, 2000; Jinno-Oue *et al.*, 2001; Saphire *et al.*, 2001). Because such binding proteins contribute to infections, it can be difficult to unambiguously distinguish them from receptors that directly mediate the membrane fusion process, especially for viruses that bind to their authentic receptors only weakly (e.g., for retroviruses, in the cases of FeLV-T or polytropic MuLVs; Anderson *et al.*, 2000; Marin *et al.*, 1999; Temin, 1988). We emphasize this because pathogenic variants of different animal viruses have often been associated with abilities to bind to apparently novel cell surface components, and it has sometimes been inferred that the viruses have switched their receptor specificities. In these instances, it has generally not been established that the cell surface binding components are receptors that directly mediate infections. In the case of Ebola, for example, the receptor(s) that mediates its entry has yet to be definitively identified. C-type lectins such as DC-SIGN and DC-SIGNR are thought to serve as adherence factors for Ebola or Marburg virus (Marzi *et al.*, 2006; Matsuno *et al.*, 2010). Other plasma membrane-associated proteins have been implicated in EBOV uptake, including folate receptor alpha and the tyrosine kinase receptor Axl (Chan *et al.*, 2001; Shimojima *et al.*, 2006, 2007; Sinn *et al.*, 2003), but the physical interaction of EBOV GP and these proteins has not been demonstrated, and cells that do not express these proteins are permissive for EBOV GP-mediated virion uptake. Some previous studies have implicated the actin cytoskeleton in EBOV entry, where agents such as cytochalasin D and swinholide A that impair microfilament function, inhibited GP-mediated entry (Yonezawa *et al.*, 2005). Similarly, VSV was shown to bind ubiquitously to cells via phosphatidylserine (PS) (Schlegel *et al.*, 1983). However, a more recent study reports that PS is not a receptor for VSV, as no correlation was found between cell surface PS levels and VSV infection, and annexin V, which specifically binds PS, did not inhibit infection of VSV (Coil and Miller, 2004). Therefore, the cell surface receptors for VSV have not been identified, but it is generally thought that binding via the G-protein is rather unspecific and involves negative charges on the plasma membrane (Carneiro *et al.*, 2006; Coil and Miller, 2005).

Adsorption of viruses onto cultured cells from the medium is usually a very slow and inefficient process, principally because of the slow rates of their diffusion into contact with the cell surfaces (Allison and Valentine, 1960; Andreadis *et al.*, 2000). In general, the rate of contact cannot be significantly enhanced by mixing or stirring, because the boundary layer of the relatively stationary fluid that surrounds walls or other large objects (e.g., cells) in flowing liquids is substantial compared with the rate of virus diffusion, hence stirring does not increase the concentration of virus surrounding this boundary zone (Allison and Valentine, 1960). In the case of retroviruses, it has become especially clear that adsorption is a severely limiting step in infection of cultured cells. In classic studies in which virus samples were incubated with cells for several hours before

washing with fresh medium and subsequently detecting the foci of infection, it was estimated that only 1/1000 or fewer of the virions in the medium were infectious. In contrast to previous interpretations, studies suggest that this low infectivity-to-virion ratio is principally caused by the inefficiency of adsorption (Andreadis *et al.*, 2000). Accordingly, serial incubation of a virus-containing medium for 2-h periods with sequential cell cultures results in the same titers in each of the cultures after correction for spontaneous viral decay (Kabat *et al.*, 1994). Furthermore, centrifuging the virus down onto the cultured cells (i.e., spinoculation) often increases retroviral titers by 1–2 log orders of magnitude (Bahnson *et al.*, 1995).

Studies aiming to count retrovirions adsorbed onto cell surfaces by confocal immunofluoresence microscopy or by quantitative PCR methods (Marechal *et al.*, 2001; Pizzato *et al.*, 1999, 2001) have demonstrated that receptors for viral entry are irrelevant for initial adsorption of retrovirions onto surfaces of most cells (Pizzato *et al.*, 1999, 2001). On the contrary, the initial steps of virus attachment seem to more critically depend on accessory cellular binding substances, such as heparin sulfates, integrins, or lectins, including DC-SIGN (Bounou *et al.*, 2002; Guibinga *et al.*, 2002; Jinno-Oue *et al.*, 2001; Mondor *et al.*, 1998; Saphire *et al.*, 2001). By forming multivalent weak reversible bonds with such abundant cell surface components, a virus would become efficiently bound in a manner that would allow it to "graze" until it makes appropriate contact with a true receptor (Haywood, 1994; Park *et al.*, 2000).

2. S–S shuffling

It is well known that for several viruses (Rubella togavirus, BVDV pestivirus, Newcastle disease paramyxovirus, HIV lentivirus, mouse hepatitis coronavirus (MHV)), rearrangements of the thiol content and of the disulfide bridges, induced by thioredoxine or protein disulfide isomerase (PDI), are essential to induce some big conformational changes necessary for the membrane fusion (Fenouillet *et al.*, 2007).

Interestingly, the MLV and HTLV retroviruses developed an "internal" oxydoreduction activity by adapting a catalytic motif involved in disulfure bridge isomerization. The two SU and TM subunits can be linked in either a covalent or noncovalent manner. For HIV-1, the existence of the soluble gp120 protein indicates a noncovalent link between SU and TM (Kowalski *et al.*, 1987). However, for most other retroviruses, a covalent link was described at one stage. In all cases, with the exception of MMTV and JSRV, a disulfide bond between the SU and the TM is formed between the highly conserved CX6CC motif of the TM and the CXXC of the SU (Pinter *et al.*, 1997; Schulz *et al.*, 1992; Sitbon *et al.*, 1991). This CXXC motif is extremely rare in cellular proteins and is

similar to a motif found in the catalytic site of enzymes involved in thiol isomerization, like PDI or thioredoxin (Pinter *et al.*, 1997; Sanders, 2000). This motif in the SU has been shown to be part of an autocatalytic isomerization function of SU to destroy the initial bond between SU and TM generated during Env synthesis and create an intra-SU bond inside the CXXC motif (Li *et al.*, 2008; Wallin *et al.*, 2004). This disulfide bond isomerization is crucial for the fusogenicity of γ-retrovirus (MLV) (Fenouillet *et al.*, 2007).

3. Fusion activation by proteases

Some viruses use the protease activity of particular cellular enzymes localized in the endosomes or at the cellular surface for activating their EnvGP. The Ebola filovirus (for which the GP1–GP2 envelope glycoprotein is cleaved by the furine in the producer cells) (Chandran *et al.*, 2005; Schornberg *et al.*, 2006), the HeV (Pager and Dutch, 2005), NiV (Diederich *et al.*, 2009), or the SARS-CoV and MHV-2 coronaviruses (for which the S spikes is not cleaved in the producer cells) (Huang *et al.*, 2006; Qiu *et al.*, 2006; Simmons *et al.*, 2005) require the cysteins lysosomal proteases, the L or B cathepsine (CatL or CatB) for their entry process. After virus uptake following angiotensin-converting enzyme 2 receptor binding, cathepsin L-mediated proteolysis induces conformational changes in the SARS-CoV S glycoprotein to trigger the endosomal membrane fusion process (Simmons *et al.*, 2005). Likewise, cleavage of the Ebola glycoprotein by CatL cleavage removes a glycosylated glycan cap and mucin-like domain (MUC domain) and exposes the conserved core residues implicated in receptor binding (Chandran *et al.*, 2005; Hood *et al.*, 2010; Lee *et al.*, 2008; Schornberg *et al.*, 2006). Entry of this virus is pH-dependent and associated with the cleavage of GP by proteases, including CatL and/or CatB, in the endosome or cell membrane, which is required for entry into the host cell. However, the precise role of the cleavage of Ebola envelope glycoprotein, which is already cleaved intracellularly during its exit, is uncertain. The cleavage of the GP to a stable form of 18 kDa of GP1 may increase binding, suggesting that the cleavage facilitates the interaction with a cellular receptor (Dube *et al.*, 2009; Kaletsky *et al.*, 2007). Another possibility is that the CatB cleavage is required to facilitate the triggering of viral membrane fusion by destabilizing the prefusion conformation of Ebola envelope glycoprotein (Wong *et al.*, 2010).

The cathepsins comprise a family of lysosomal protease enzymes whose primary function (i.e., protein degradation) plays a critical role in normal cellular homeostasis. Cathepsin L is one of the 11 members of human lysosomal cysteine proteases (i.e., B, C, F, H, K, L, O, S, V, W, and X) that fall in the C1 family (papain family) of the CA clan (Rossi *et al.*, 2004). These enzymes were traditionally linked to nonspecific proteolytic activity within lysosomes. More

recently, cathepsin L has been implicated in regulatory events relating to cancer, diabetes, immunological responses, degradation of the articular cartilage matrix, and other pathological processes (Vasiljeva *et al.*, 2007), including osteoporosis, rheumatoid arthritis, and tumor metastasis (Palermo and Joyce, 2008).

Interestingly, several host cell proteases appear to be able to prime fusion activation in the case of SARS-CoV, including cathepsin L, trypsin, factor Xa, thermolysin, plasmin, TMPRSS11a, and elastase. The proteolytic cleavage events in SARS-CoV S that lead to membrane fusion occur both at the S1/S2 boundary and adjacent to a fusion peptide in the S2 domain (Belouzard *et al.*, 2009). Elastase-mediated activation of SARS-CoV was originally reported by Taguchi and coworkers, and it has been proposed that elastase may have important implications for viral pathogenesis (Matsuyama *et al.*, 2005). Elastase is known to be secreted by neutrophils as part of an inflammatory response to a viral infection and is also produced by opportunistic bacteria (e.g., *Pseudomonas aeruginosa*) that can colonize virally infected respiratory tissue. As such, it has been considered that elastase-mediated activation of SARS-CoV might be an important factor in the severe pneumonia seen in SARS-CoV-infected patients (Belouzard *et al.*, 2010).

In conclusion, in order to better classify the receptors, the receptors must be differentiated according to their precise function: those that permit a nonspecific adsorption, such as the glycosaminoglycans (used by Dengue, TBE, HSV-1), the type C lectin receptors, such as L-SIGN, DC-SIGN, the hMGL, the LSECtin, and the asialoglycoprotein receptor (used by HCV, Ebola, Marburg); those that permit a specific adsorption receptor (binding receptor) allowing the sorting (toward particular endosomes and intracytoplasmic compartment) and the initial conformational changes (such as the CD4 to HIV, the integrins or the laminin receptor for the Dengue, Sindbis, or Lassa viruses); and finally, those that permit the exposition of the domains implicated in the destabilization of the membrane (like the fusion peptide) and are the latest receptors to act (such as the HIV coreceptors). Therefore, studying the kinetics of the conformation changes of the EnvGP and the kinetics of action and utilization of the receptors is essential to accurately categorize the receptors (nonspecific adsorption receptor, receptor of binding, receptor of fusion).

All the cellular proteins that allow the exposition, localization, and the trafficking of these receptors and/or endosomes should be considered as cofactors. With all the siRNA screens that are coming out, the list of such cofactors is dramatically increasing.

B. Use of multiple receptors—Receptor switch

The use of different receptors often correlates with the need of a virus to overcome barriers existing in the cell type or tissue that they infect. One well-studied example is the binding of Coxsackievirus B to decay-accelerating factor

(DAF) in the apical surface of epithelial cells, and subsequently to the Coxsackievirus and adenovirus receptor (CAR), which is localized in the tight junction region. DAF helps to bring the virus to the tight junctions, and CAR induces a conformational change and promotes endocytosis (Coyne and Bergelson, 2006).

Almost all animal viruses use receptors exclusively containing a single TM sequence (see Table 4.1). In striking contrast, cell surface receptors for γ-retroviruses have multiple transmembrane (TM) sequences, compatible with their identification in known instances as transporters for important solutes. Similarly, hepatitis C virus, in addition to LDL receptor, exclusively uses multitransmembrane receptors:the Scavenger receptor class B type 1 (SRB1 with two transmembrane domains), the tetraspanin receptors CD81 and Claudins (4 TMs), and another tight junction protein Occludin-1 (4 transmembrane domains; Ploss and Rice, 2009).

Another surprise is that some viruses, including many γ-retroviruses, use not just one receptor but pairs of closely related receptors as alternatives (Tailor *et al.*, 2003). This appears to have enhanced viral survival by severely limiting the likelihood of host escape mutations. All of the receptors used by γ-retroviruses contain hypervariable regions that are often heavily glycosylated and that control the viral host range properties, consistent with the idea that these sequences are battlegrounds of virus–host coevolution. However, in contrast to previous assumptions, it is probable that γ-retroviruses have adapted to recognize conserved sites that are important for the receptor's natural function and that the hypervariable sequences have been elaborated by the hosts as defense bulwarks surrounding the conserved viral attachment sites.

The fact that all virus groups have been severely limited throughout evolution in the types of receptors they can employ, may initially appear inconsistent with evidence that some viruses can switch their receptor specificities with apparent ease. This has been most dramatically suggested by shifts of influenza A viruses between animal reservoirs, which involve single amino acid changes in the viral hemagglutinin, enabling recognition of different sialic acid structures (e.g., *N*-acetyl or *N*-glycolyl neuraminic acids in α2,6 or α2,3 linkages to galactose) that predominate in the different host species (Baranowski *et al.*, 2001; Gambaryan *et al.*, 2005; Skehel and Wiley, 2000). Similarly, slight changes in specificity for receptors accompanied the emergence, in 1978, of the canine parvovirus (Parker *et al.*, 2001). However, these are small shifts in receptor specificities rather than global jumps to dissimilar receptors. Similar slight shifts are involved in the change from the CCR5 to CXCR4 coreceptor usage during AIDS progression (Scarlatti *et al.*, 1997). Small shifts in usages of highly similar receptors have also been reported during cell culture selections of subgroup B, D, and E avian leukosis viruses that all use polymorphic variants of the same TVB receptor (Taplitz and Coffin, 1997) and during cell culture selections of HIV-1 variants (Platt *et al.*, 1998). Therefore, despite the rarity of receptor repertoire

expansions throughout millions of years of retrovirus evolution, limited switches can occur within single infected animals. For example, there is an evolution of coreceptor usage in HIV-1/AIDS, some *in vivo* adaptations of ecotropic MuLVs and formation of polytropic MuLVs, and some evolution of altered receptor usages in domestic cats infected with FeLV-A (Tailor *et al.*, 2003).

Several viruses have been reported to use multiple alternative receptors or even alternative pathways for infection of cells. For example, MV isolates appear to be capable of using CD46 or SLAM, which both contain single TM domains (Baranowski *et al.*, 2001; Oldstone *et al.*, 1999; Tatsuo *et al.*, 2000). Complex viruses such as herpesviruses that contain several distinct EnvGP are also typically able to bind to several cell surface components (Baranowski *et al.*, 2001; Borza and Hutt-Fletcher, 2002). The foot-and-mouth disease picornavirus (FMDV) may also use multiple receptors, including heparan sulfates and integrins, and may, in addition, be able to invade cells via immunoglobulin Fc receptors when the virus is coated with antibodies (Baranowski *et al.*, 2001; Mason *et al.*, 1994). This alternative entry route is also used by the dengue flavivirus, which may explain the extremely strong pathogenesis that occurs when it reinfects previously exposed individuals (Baranowski *et al.*, 2001). In the case of FMDV, it has not been established whether heparin sulfate is a true receptor that directly mediates infection or merely a binding factor that influences infection indirectly by enhancing virus adsorption. HIV-1 infections are also strongly stimulated by accessory cell surface binding components including heparan sulfates, glycolipids, and DC-SIGN (Bounou *et al.*, 2002; Geijtenbeek *et al.*, 2000; Pohlmann *et al.*, 2001). Similarly, a paralysis-inducing neurotropic variant of Friend MuLV binds more strongly than the parental virus to heparan sulfate, and thereby becomes more infectious for brain capillary endothelial cells while still remaining dependent on the CAT1 receptor (Jinno-Oue *et al.*, 2001). These examples illustrate how changes in affinities for accessory binding substances can dramatically alter cellular tropisms and pathogenesis of viruses, and why it has often been difficult to distinguish such accessory binding factors from true receptors or coreceptors that are essential for infections. On the basis of these considerations, we believe that the available evidence strongly supports our proposal that all virus groups have been severely constrained in the types of receptors they can employ for infection of cells. However, some viruses have evolved several pathways for infection, and viruses such as HIV-1 have evolved distinct sites in a single SU glycoprotein for recognition of dissimilar receptors and coreceptors.

C. Separation of the binding and fusion functions

The complexity of EnvGP is variable. An example of a very simple fusion protein is the FAST proteins of Orthoreovirus that do not belong, however, to the family of the enveloped virus (Barry *et al.*, 2010; Shmulevitz and Duncan, 2000). The

Orthoreovirus genus includes some no-enveloped viruses that cause the cell–cell fusion of infected cells. This fusion activity is due to the small no-structural membrane viral proteins named FAST proteins (fusion-associated small transmembrane protein), in size ranging between ~10 and 15 kDa. The FAST protein ectodomains are very small, with extreme cases of only 20 residues, and contain hydrophobic short regions with/without acid properties, and a myristoylation site. Though their small size challenges their ability to form a hairpin structure, these FAST proteins expressed alone are sufficient to induce the membrane fusion (Barry *et al.*, 2010; Shmulevitz and Duncan, 2000). Surprisingly, they are able to traffic as far as the plasma membrane without inducing intracellular disorder. Indeed, by opposition, the expression of retroviral TM or orthomyxovirus HA2 alone does not lead to cell surface membrane expression, and these subunits are blocked intracellularly. The fusion induced by the FAST protein is very broad, which questions their use of a particular protein receptor. Thus, some fusion proteins are simple and possess solely the fusion function, and others comprise several domains in addition to the domain involved in fusion. The function of some of these domains has remained more or less independent from the fusion domain, and sometimes, they can be naturally separated in different proteins (i.e., H and F from paramyxoviruses) or experimentally on different fragments, as explain below. Such is the case of the γ-retroviruses for which the function of binding to the receptor can be separated from the function of fusion. This line of inquiry was initiated by the studies of Bae *et al.* (1997) relating to a conserved PHQ motif that occurs near the amino-terminal ends of SU glycoproteins in all γ-retroviruses. Mutation of this PHQ motif blocked membrane fusion but had no effect on receptor attachment. Subsequently, we discovered that noninfectious γ-retrovirions lacking this histidine could be transactivated by addition of a soluble SU or an amino-terminal fragment of SU, called the receptor-binding domain (RBD), to the cultured cells (Lavillette *et al.*, 2000). Interestingly, studies by Overbaugh and coworkers have demonstrated that similar transactivation processes can occur in natural infections by γ-retroviruses. Specifically, they found that infections by the immunosuppressive FeLV-T virus, which has a Pro in place of His in its PHQ motif, require transactivation either by a soluble FeLV-B-related SU glycoprotein termed FELIX that is endogenously expressed in cat T cells or by an FeLV-B SU glycoprotein (Anderson *et al.*, 2000). This activating process parallels that of herpes simplex virus for the transmission of a fusogenic signal among the EnvGP of the herpes simplex virus on receptor binding by glycoprotein gD. The soluble gD ectodomain has been shown to allow entry of engineered HSV-1 virus particles that lack gD (i.e., gD-null mutants; Cocchi *et al.*, 2004). The evidence reviewed provides very strong support for the hypothesis that attachment of viruses to their receptors initiates a pathway that obligatorily contains intermediate steps. These intermediate steps very likely include viral association with multiple receptors,

cooperative conformational changes within Env glycoprotein, and cross-talk between Env on the viral surfaces. In the case of the retroviruses, this evidence suggests that virus binding to receptors does not directly induce irreversible structural changes in SU–TM complexes as was previously believed. Rather, it implies that the binding to receptors induces SU–SU interactions that are prerequisites for later steps in a highly coordinated membrane fusion pathway. We anticipate that similar intermediate steps are likely to be involved in infections by other groups of retroviruses and perhaps in infections by other membrane-enveloped viruses.

Other viruses can be activated by soluble receptors that, by interacting with the envelope glycoprotein, induce some of the conformational changes necessary to trigger fusion. This example indicates that binding and fusion steps can be separated and can take place at different locations. In these cases, MHV, avian retrovirus ASLV, and the herpes simplex 1 (HSV-1) can infect some cells that do not harbor binding receptors provided that the soluble form is present in the infection media (the soluble CEACAM1 receptor for MHVR, the soluble Tva receptor for ASLV, and the soluble nectin-1 for HSV-1; Kwon *et al.*, 2006; Matsuyama and Taguchi, 2002; Mothes *et al.*, 2000).

IV. BASIC AND TRANSLATIONAL RESEARCH EXPLOITING ENTRY PROPERTIES OF VIRUSES

A. Tropism properties and use of pseudoparticles in gene therapy

Vectors derived from retroviruses such as lentiviruses and oncoretroviruses are probably among the most suitable tools to achieve long-term gene transfer, since they allow stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors should be the preferred gene-delivery vehicles over vectors derived from oncoretroviruses (MLV) since, in contrast to the latter, they can transduce nonproliferating target cells. Moreover, lentiviral vectors that have the capacity to deliver transgenes into specific tissues are expected to be of great value for various gene transfer approaches *in vivo* (Frecha *et al.*, 2008b). To achieve such *in vivo* gene transfer, innovative approaches have been developed to upgrade lentiviral vectors for tissue or cell targeting and which have potential for *in vivo* gene delivery. One strategy is to develop vectors that harbor EnvGP with selective tropisms. Vectors derived from retroviruses offer particularly flexible properties in gene transfer applications, given the numerous possible associations of various viral surface glycoproteins (determining cell tropism) with viral cores. Selective tropisms were achieved by taking advantage of the natural tropisms of EnvGP from other membrane-enveloped viruses. For instance, the use of surface glycoproteins derived from viruses that cause lung infection and

infect via the airway epithelia, such as Ebola virus or Influenza virus, may prove useful for gene therapy of the human airway (Kobinger *et al.*, 2001). Exclusive transduction of retinal pigmented epithelium could be achieved following subretinal inoculations of some vector pseudotypes in rat eyes (Duisit *et al.*, 2002). High transgene expression was detected in dermal fibroblasts transduced with VSV-G-, EboZ-, or MuLV-pseudotyped HIV vector and effectively targeted quiescent epidermal stem cells which underwent terminal differentiation resulting in transgene expression in their progenies (Hachiya *et al.*, 2007). Importantly, several viral EnvGP target lentiviral vector to the CNS such as rabies (Wong *et al.*, 2004), mokola (Watson *et al.*, 2002; Wong *et al.*, 2004), lymphocytic choriomeningitis virus envelope (LCMV) (Miletic *et al.*, 2004; Stein *et al.*, 2005), or Ross River (Kang *et al.*, 2002) viral EnvGP that even permit transduction of specific cell types within the CNS. Likewise, screening of a large panel of pseudotyped vectors established the superiority of the gibbon ape leukemia virus (GALV) (Sandrin *et al.*, 2002; Stitz *et al.*, 2000) and the cat endogenous retroviral-modified glycoproteins (RD114) (Sandrin *et al.*, 2002) for transduction of progenitor and differentiated hematopoietic cells. Recently, a new LV carrying the MV EnvGP on its surface was able to overcome vector restrictions in both quiescent T and B cells (Frecha *et al.*, 2008a, 2009). Importantly, naive as well as memory T and B cells were efficiently transduced, while no apparent activation, cell-cycle entry, or phenotypic switching were detected, opening the door to a multitude of gene therapy and immunotherapy applications. Vectors derived from HIV pseudotyped with Sendai virus fusion protein F (Kowolik and Yee, 2002) or E1E2 from hepatitis C virus (Bartosch *et al.*, 2003), and such vectors are able to transduce human hepatoma cells and primary human hepatocytes efficiently, although they are unable to enter nonliver cells. The GP64 glycoprotein from baculovirus *Autographa californica* multinuclear polyhedrosis virus pseudotyped FIV efficiently and also showed excellent hepatocyte tropism (Kang *et al.*, 2005).

B. Identification of viral cell entry receptors using pseudoparticles

The screening of cDNA libraries has emerged as a powerful tool to identify and clone viral entry receptors. It is an alternative to the use of human/Chinese hamster radiation hybrid panels of cells. In order to clone an unknown receptor by complementation screens, cDNA from a cell permissive to infection by a certain virus is introduced into a nonpermissive cell. As some recessive cell lines are poorly transfectable, the use of pseudoparticles provides a good tool to transduce the cDNA library for this cloning strategy. A retroviral cDNA library approach, involving transfer and expression of cDNAs from highly infectable cells to nonpermissive cells, has been used to clone and identify the MuLV polytropic X-receptor (Battini *et al.*, 1999; Tailor *et al.*, 1999a; Yang *et al.*, 1999),

the RD114 ASCT2 receptor (Rasko *et al.*, 1999; Tailor *et al.*, 1999b), FeLV-C FLVCR1 receptor from human and domestic cat cDNA libraries (Quigley *et al.*, 2000; Tailor *et al.*, 1999c), and two closely related human proteins, PHuR-A1 and PHuR-A2, that function as receptors for PERV-A (Ericsson *et al.*, 2003). Briefly, when a cell line that is not susceptible to a particular pseudotype retrovirus vector harboring an envelope for which the receptor is not known, a cDNA library from a cell line highly susceptible to transduction, was constructed by cloning the cDNA into a retroviral expression vector. Afterward, the cDNA retroviral expression library was transduced into nonsusceptible cells by infection at a relatively low multiplicity of infection so that the majority of infectants would contain single-copy provirus inserts. The library-containing cells were then screened for susceptibility to pseudotype vector transduction through selection of drug-resistant cells after exposure to the vector carrying a resistance gene. Of drug-resistant clones obtained from the primary screen and using PCR primers specific for vector sequences, cDNA products from clones with conferred susceptibility were identified after nested PCR was performed on DNA extracted from reinfectable clones.

However, for many viruses, initial attempts using a retroviral cDNA library were unsuccessful due to an inherent background of nonspecific infection with pseudoparticles. In fact, no cell line was completely nonpermissive to even "no envelope" pseudoparticles bearing no viral envelope proteins, indicating the existence of nonspecific uptake mechanisms. In the screens, this resulted in a high background of drug-resistant colonies, independent of glycoprotein-mediated cell entry. Thus, unless the entry factor cDNA was highly represented in the library, a single round of transduction/challenge would not suffice. To deal with the high background observed in screens, methods that would allow multiple rounds of selection and enrichment have been developed. Recently, the use of a cyclic packaging rescue (CPR) system using non-self-inactivating vectors has been shown to increase the efficiency of receptor cloning with powerful iterative screening methods (Evans *et al.*, 2007; Ploss *et al.*, 2009). Most retroviral vectors commonly used for gene-delivery applications are self-inactivating vectors that contain deletions in the long terminal repeat (LTR) elements. No packaging competent retroviral RNA transcripts are generated from such integrated proviruses; instead, transgene expression is driven by an internal nonretroviral promoter. In contrast, if the cDNA library is constructed in a provirus that retains the complete LTR elements, the retroviral promoter is active in transduced cells and a full-length viral RNA is expressed. Expression of the packaging components, gag-pol and vesicular stomatitis virus VSV-G envelope (that will efficiently infect recessive cell lines), in these cells allows packaging of the RNA into pseudoparticles capable of transducing naive cells. This approach, termed CPR (Bhattacharya *et al.*, 2002; Koh *et al.*, 2002), allows retrieval of the library after selection has been performed, followed by

transduction of a naive cell population, concluded by a new round of selection. This process can be repeated sequentially for an unlimited number of selection/enrichment steps.

For an additional level of selection, different challenge virus genomes can be used, each encoding a different drug-selectable marker (e.g., puromycin (PuroR) or zeocin (ZeoR) resistance), in self-inactivating retroviruses. Thus, after challenge and selection of a library-transduced population with one pseudoparticle-packaged selection cassette (e.g., PuroR), the population can be pooled and rechallenged with the second-selectable pseudovirus (e.g., ZeoR). Then, during CPR, only the full-length retroviral transcripts from the non-self-inactivating provirus that encodes the library but not the self-inactivating challenge virus genomes are repackaged and transferred to the naive cell population. This enables researchers to perform multiple rounds of selection, thereby overcoming the background of nonspecific pseudoparticle uptake. Moreover, using this scheme, underrepresented cDNA will be enriched. Once the final round of selection of pseudotype-susceptible cells has been achieved, genomic DNA can be prepared from selected clones and used as a template in a PCR across the provirus cDNA-cloning site.

The nonpermissive target cell line for the cDNA screen adheres to several stringent criteria. As stated above, the primary requirement is that (1) to minimize nonspecific background, cell lines with minimal uptake of pseudoparticle of interest and "no envelope" pseudoparticles were preferred. (2) To ensure that nonpermissiveness was a phenotype due to the lack of a pseudoparticle-specific entry factor(s) rather than poor infection by pseudotypes in general, chosen cell lines should be highly permissive to an unrelated pseudoparticle (VSVGpp, rhabdovirus) infection. In addition, this also ensures that the target cell line would be easily transduced with the cDNA library. (3) For selection, candidate cell lines also had to be susceptible to the desired drug selections. (4) To perform multiple rounds of screening involving CPR, the ideal cell line had to demonstrate this method well and be highly transfectable. (5) Finally, to facilitate the screen, the chosen cell line needed to be relatively fast growing and clone efficiently.

C. Determining the endocytosis pathway of entry and the different cell proteins involved in entry by RNA interference screens

To gain insights into virus entry, it is necessary to examine several inhibitors of pathway-mediated endocytosis in terms of their role in blocking infection mediated by pseudotypes with different EnvGP. An advantage of pseudoparticles is that the entry process of pathogens of BSL4 can be studied in BSL2 conditions. For example, to gain insights into Ebola virus entry, inhibitors against different endocytosis pathways have been examined for their ability to block infection

mediated by HIV pseudotyped with the Ebola EnvGP (Bhattacharyya *et al.*, 2010). The use of control pseudoparticles (e.g., pseudotyped with Vesicular Stomatitis Virus EnvGP (VSV G)) can be used as controls to assess cell viability and specificity of inhibition. Inhibition of clathrin function traditionally relied on three principal approaches: drugs that inhibit acidification of endosomes (such as BafA1, a specific, nonreversible endosomal proton pump inhibitor), as well as commonly used lysosomotropic agents (such as ammonium chloride (NH_4Cl) and chloroquine); potassium depletion; and finally, treatment of cells with brefeldin A (BFA) or chlorpromazine (Sieczkarski and Whittaker, 2002). As such, these drugs have multiple effects on cell function, and their use to inhibit virus infection should be treated with some caution. Thanks to pseudotypes, some other studies have implicated the actin cytoskeleton in Ebola virus entry, where agents such as cytochalasin D and swinholide A that impair microfilament function were shown to inhibit EnvGP-mediated entry (Yonezawa *et al.*, 2005). Ebola enters cells through a low-pH-dependent, endocytosis-mediated process. A large body of evidence indicates that Ebola viruses enter cells by clathrin-mediated endocytosis (Sanchez, 2007), but lipid raft-associated, caveolin-mediated endocytosis has also been proposed as an alternative mechanism of Ebola virus uptake (Empig and Goldsmith, 2002). Low-pH events lead to cathepsin-dependent cleavage of Ebola virus EnvGP that is required for productive uptake of the virus (Chandran *et al.*, 2005; Kaletsky *et al.*, 2007; Schornberg *et al.*, 2006). Other low-pH-dependent events have been postulated to be required as well (Schornberg *et al.*, 2006). Furthermore, Ebola virus likely uses a Rho-mediated pathway, as is seen in VSV virus, suggesting that this may be a route of entry utilized by many different viruses (Quinn *et al.*, 2009).

More recently, proteins interfering with endocytosis, such as the use of dominant-negative Eps15, or RNA interference (RNAi) have been developed, and such approaches target the different pathways with higher specificity (Mercer and Helenius, 2009; Mercer *et al.*, 2010b). The RNAi approach allows researchers to perform screens to identify previously unrecognized host factors that are required for viral replication. RNAi screens rely on either short interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) to knock down the function of a particular gene in a cell. Researchers can then infect the cells with specific viruses and monitor levels of viral replication. If viral replication is reduced, then the knocked-down gene might be necessary for the virus to replicate itself or function within the host cell.

Some small-scale screens have been developed using wild-type viruses (Kolokoltsov *et al.*, 2007) or pseudoparticles (Trotard *et al.*, 2009). Pseudoparticles can be exploited to focus a siRNA screen specifically on the entry step of a virus. Indeed, only the entry steps are governed by the EnvGP, whereas all the uncoating, integration, and expression steps of the transgene depend on

retroviral proteins. Having said that, this suffers an inherent drawback in that some steps are dependent on retroviral proteins and therefore limits the scope of the screen. One limitation to the siRNA screen is specificity and cell toxicity, which can be overcome by the use of pseudotypes. Indeed, a siRNA screen can be done with different pseudotypes in parallel. If, contrary to the pseudotypes of interest, some pseudotypes are not affected by siRNA, this indicates that the siRNA specifically inhibits the virus of interest and, moreover, that the siRNA is not toxic. To better characterize the entry pathway of the hepatitis C virus, a small interfering RNA library dedicated to membrane trafficking and remodeling was screened in the context of the Huh-7.5.1 liver cell line cells infected by HCV pseudoparticles (HCVpp) (Trotard *et al.*, 2009). Results showed that the downregulation of different factors implicated in clathrin-mediated endocytosis inhibit HCVpp cell infection. In addition, knockdown of the phosphatidylinositol 4-kinase type III-alpha (PI4KIIIalpha) prevented infection by HCVpp, and the presence of PI4KIIIbeta in the host cells influenced their susceptibility to HCVpp infection. This library screening using pseudoparticles identified two kinases, PI4KIIIalpha and beta, as being involved in the HCV life cycle. These results have been confirmed in published works of the identification of cellular factors required for the HCV life cycle using siRNA libraries screening either over 4500 drugable genes (Ng *et al.*, 2007; Vaillancourt *et al.*, 2009), 140 cellular membrane-trafficking genes (Berger *et al.*, 2009; Coller *et al.*, 2009), kinome (Supekova *et al.*, 2008), or the entire genome (Li *et al.*, 2009; Tai *et al.*, 2009). These works are much more time- and cost-consuming, and even though pseudoparticles may appear to be an overly simple model to study and identify host factors necessary for viral infection, they are also powerful and flexible tools that have led to major discoveries, as these examples have shown.

Recently, however, in order to identify host factors necessary for viral infections, researchers are turning to genome-wide genetic screens. Indeed, the sequencing of the human genome and the emergence of technologies that allow the silencing of individual genes in cells one by one using siRNA, when combined with the use of genome-wide siRNA libraries and automated HTS methodology, allows molecular information to be gained about virtually every critical intracellular event occurring during virus infection. Within the last few years, there have been several publications describing such genome-wide siRNA screens that have been applied to virus infections in tissue-cultured cells. RNAi screens have now been preformed for several viruses, including HIV (Brass *et al.*, 2008; Konig *et al.*, 2008; Yeung *et al.*, 2009; Zhou *et al.*, 2008), West Nile Virus (Krishnan *et al.*, 2008), Influenza (Karlas *et al.*, 2010; Konig *et al.*, 2008), and, in drosophila cells, Dengue (Sessions *et al.*, 2009) and vaccinia virus (Moser *et al.*, 2010). From the hundreds of cellular factors that have been implicated in the outcome of infection, some were already known from other studies, but the majority are entirely novel, and the validation and analysis of data is ongoing

in many laboratories. However, a poor overlap between results from different screens published on the same viruses has been observed. The differences are probably linked not only to technical variations but also to the inherent risk of false positives through off-target effects and false negatives due to inefficient silencing. Cell toxicity is a common complication, as well as cell-type specificity. Following-up such screens with pseudoparticle studies is useful to validate the cell proteins that are involved in the entry step only and facilitate the analysis, compared to the use of a replication competent virus.

D. Screening and development of entry inhibitors using pseudoparticles

Resistance to individual antivirals is likely to develop for most specific therapeutics targeting particular viral proteins, thus making therapy consisting of a combination of drugs targeting different stages of the viral life cycle highly desirable. The entry process represents another series of potential targets for therapeutic intervention. It has not been extensively explored due to high-throughput experimental limitations for some viruses that require biosafety level 3 or 4 or for the viruses with no robust infection system.

Pseudoparticle infection systems, utilizing different reporter genes or proteins (i.e., firefly luciferase or GFP), can be developed for HTS of small molecule libraries, peptide libraries, or neutralizing antibodies for their entry inhibitor properties. In order to facilitate the screen, assay performance can be improved by modifying the properties of both the parental host cell line and the pseudovirus. For example, cells with improved pseudoparticle entry and cell spreading can be selected. Pseudoparticles can be improved by using EnvGP that increase infectivity either by selecting a specific variant or by expressing a human codon-optimized envelope glycoprotein sequence. Finally, the pseudoviruses can be engineered to express a human codon-optimized reporter to improve the sensitivity of the assay. Using these modifications, HTS can be developed using 96- or 384-well plates. Compounds found to inhibit pseudoparticle infection can then be counterscreened against pseudoparticles containing other variants to characterize the cross-reactivity of the molecules in a virus family. Moreover, molecules can then be counterscreened against pseudoparticles harboring EnvGP from other virus families to evaluate the specificity of the inhibitor or its large spectrum.

A wide variety of entry inhibitors, namely, peptides, chemical compounds, or antibodies, exist. They can interfere with the different steps of the entry process, such as the binding to receptors or the conformational changes of the fusion proteins, by acting on the envelope protein itself, on the acidification of the endosomes, if necessary, or on the endocytosis.

For HIV, most marketed drugs for treating AIDS are inhibitors of HIV-1 reverse transcriptase or protease enzymes, but new targets include the integrase enzyme, cell–surface interactions, membrane fusion, and also virus particle maturation and assembly (Kaushik-Basu *et al.*, 2008). Entry inhibition entails preventing HIV-1 breaching the cell, either as a strategy to prevent infection altogether or to curtail infection of new cells in an HIV-positive individual. Several strategies have proved effective in HIV-1 entry inhibition either *in vitro* (using pseudoparticles or replication competent viruses) or *in vivo*: binding to viral surface proteins gp120 and gp41, binding to human cell surface receptor CD4, and binding to human cell surface coreceptors, CCR5 and CXCR4 (Leonard and Roy, 2006; Liu *et al.*, 2007). In particular, the synthesized peptide T-20 is believed to act by binding to gp41 (Wild *et al.*, 1993, 1994) and is currently in clinical use. Yet, several more recent studies have revealed that T-20 does not block the six-helix bundle prehairpin formation (Liu *et al.*, 2005). Another peptide, C37, derived from the C-terminus of gp41 (and nearly identical to the widely reported C-peptide C34; Liu *et al.*, 2005; Root *et al.*, 2001), is also described as having strong anti-HIV entry activity due to the tight binding of the gp41 N-terminal helices. Maraviroc is a small antiretroviral compound known to be a CCR5 antagonist, which blocks R5-tropic HIV entry into CD4 cells (Hunt and Romanelli, 2009). Several studies have established that synergy can occasionally be observed when two fusion inhibitors are combined in a viral assay or *in vitro* fusion assay. For example, some synergy is observed in the combination of CCR5 antibodies with T-20 (Ji *et al.*, 2007; Murga *et al.*, 2006), the combination of small molecular antagonists of coreceptors with T-20 (Tremblay *et al.*, 2000, 2002), the combination of small molecular antagonists of CCR5 with CCR5 antibodies (Ji *et al.*, 2007; Murga *et al.*, 2006), and the combination of small molecular antagonists of CCR5 with chemokines (Murga *et al.*, 2006; Tremblay *et al.*, 2002).

In the case of HCV, entry into hepatocytes is a multistep process, involving at least four cellular receptors, leading to virion endocytosis and fusion of the viral and cellular membranes. Unlike the HCV replication process, these steps have not been thoroughly exploited as targets for antiviral intervention. Recently, with the development of HCVpp and the JFH1 infectious molecular clone, it has become possible to test drugs against entry. *In vitro*, proof-of-concept studies for inhibiting the HCV entry process have been demonstrated using cyanovirin-N that targets the N-linked glycans of the viral envelope proteins and prevents E2–CD81 interaction (Helle *et al.*, 2006), neutralizing antibodies directed against the HCV E1 and E2 proteins (Broering *et al.*, 2009; Habersetzer *et al.*, 1998; Keck *et al.*, 2008; Law *et al.*, 2008; Owsianka *et al.*, 2005; Perotti *et al.*, 2008; Schofield *et al.*, 2005; Yu *et al.*, 2004), antibodies against cellular receptors CD81 (Bartosch *et al.*, 2003; Cormier *et al.*, 2004; Lavillette *et al.*, 2005; Molina *et al.*, 2008), SR-BI (Catanese *et al.*, 2007; Zeisel *et al.*, 2007), Claudin-1

(Krieger *et al.*, 2010), and agents that block endosomal acidification (Bartosch *et al.*, 2003; Hsu *et al.*, 2003; Koutsoudakis *et al.*, 2006; Lavillette *et al.*, 2005). *In vivo* studies using humanized trimera mice model or human liver-u-PA-SCID mice have also demonstrated prophylactic efficacy of monoclonal anti-E2 (Eren *et al.*, 2006) and anti-CD81 antibodies (Meuleman *et al.*, 2008), respectively. Some broad-spectrum antiviral drugs have also been tested (Pecheur *et al.*, 2007), but more recent studies have used the HCVpp system in order to undertake a screening campaign that led to the discovery of a class of small molecule HCV-specific inhibitors consisting of several structurally related compounds defined by a common triazine core (Baldick *et al.*, 2010). Inhibition of entry was confirmed by using time-of-addition experiments to demonstrate that inhibitor activity is confined to the first 3 h of infection, with inhibition occurring postattachment and closely linked to the inhibition kinetics of the endosomal acidification inhibitor bafilomycin.

Pseudoparticles can also be used to identify the mode of action of molecules identified using a replication competent virus in a cell-based screening system involving multiple rounds of infection in a 96-well format. After analysis of a publicly available library of 446 clinically approved drugs, the impact of 33 identified compounds on viral entry was tested using HCVpp infection to recapitulate HCV particle adsorption, internalization, and viral envelope-mediated fusion (Gastaminza *et al.*, 2010). Many of the candidates were lysosomotropic compounds that inhibited HCV entry with differential efficacy.

Both pseudotype (Larson *et al.*, 2008) and infectious virus screening (Bolken *et al.*, 2006) identified broadly active arenavirus entry inhibitors. Isolation and mapping of resistant viruses, as well as chimeras between sensitive and resistant strains, mapped the target of activity to the GP2 subunit of the G envelope protein complex, specifically to the interface between the C-terminal stem and TMD domains. Mechanistic studies showed that these arenavirus inhibitors prevented low-pH-induced fusion by blocking reorganization between the GP2 stem with N-terminal domains of the G protein complex (York *et al.*, 2008).

Another alternative for developing entry inhibitor compounds is to target endosomal protease necessary for entry of certain viruses. Inhibitors of cathepsin L block viral entry of severe acute respiratory syndrome coronavirus (SARS-CoV) and Ebola virus and impair conversion of HeV glycoprotein into the mature, active form (Chandran *et al.*, 2005; Pager and Dutch, 2005; Simmons *et al.*, 2005). With respect to the development of antiviral agents, inhibitors of human cathepsin L are not subject to resistance arising from rapid mutations of the viral genome (Shah *et al.*, 2010), making Cathepsin L an attractive target for drug development. HTS for cathepsin L inhibitors identified a novel thiocarbazate compound exhibiting potent inhibition against cathepsin L (Beavers *et al.*, 2008; Myers *et al.*, 2008; Shah *et al.*, 2008). This compound prevented 293T cell

infection by the Ebola and SARS-CoV pseudotypes, respectively. In addition, the thiocarbazate inhibited *in vitro* propagation of malaria parasite *Plasmodium falciparum* and inhibited *Leishmania* major (Shah *et al.*, 2008).

Finally, another natural entry inhibitor is provided by monoclonal neutralizing antibodies. Many monoclonal antibodies can be isolated from immortalized B-cells recovered from patients or from mice hybridomas following immunization. The antibodies can be selected by ELISA assay using soluble envelope proteins or pseudoparticles. The neutralizing potential of these antibodies can be easily screened using pseudoparticles with high-throughput infection assays, and the inhibitory effect can be verified with replicating viruses. In the case of HCV, using an antibody antigen-binding fragment phagedisplay library generated from a donor chronically infected with HCV, of 115 clones showing specific binding to HCV E2 glycoprotein, 5 monoclonal antibodies presented neutralizing activities against cell-culture HCV (HCVcc), JFH-1 virus, and a panel of HCVpp displaying E1–E2 from diverse genotypes (Law *et al.*, 2008). Overall, neutralizing antibodies can inhibit the different steps of the entry process from binding to membrane fusion by targeting the domains involved in this step or by limiting the conformational changes of the envelope complex. Interestingly, one study has recently highlighted the feasibility of targeting short-lived envelope glycoprotein intermediates for inhibition of membrane fusion using monoclonal antibodies (York *et al.*, 2010). This action is very similar to the peptides against HIV membrane fusion on the market. Such strategies to effectively target fusion peptide function in the endosome may lead to the discovery of novel classes of antiviral agents, and screens using pseudoparticles will provide an easily wielded system to identify such infrequent antibodies.

V. CONCLUSIONS

All viruses have developed varied mechanisms to reach the same goal. They vary greatly in structure, but all seem to have a common mechanism of action, in which a ligand-triggered large-scale conformational change in the fusion protein is coupled to apposition and merger of the two bilayers. In spite of the different mechanisms to activate the fusion peptide, fusion proteins are distributed into three classes based on their structural homologies. Future experiments must aim to elucidate the molecular mechanisms and the dynamics of the conformational changes driving virus entry. This will require the development of new approaches to study the rapid conformational changes of a small number of membrane interacting protein molecules and domains. A more realistic goal is the determination of all the structures of proteins that mediate the entry of all human viruses, either at a prebinding or postfusion stage.

Another challenge will be the identification of the cognate cellular receptors. Identification of all the cellular receptors for human viruses would be an important contribution to our understanding of virus tropism and pathogenesis. Once known, the role of receptors in entry, as a specific or nonspecific binding factor, or as receptors needed for conformational changes, or for routing the virus to the right compartment, will have to be established. Moreover, for most viruses, the EnvGP appear to function in an autonomous manner and can permit fusion without a requirement for receptors at acidic pH. Thus, the role of varied receptors remains enigmatic for many viruses. It is difficult to predict the roles of a receptor in fusion, sorting and routing the virus toward a particular favorable compartment in the pursuit of the infectious cycle based upon its family and its structure. The activation process of EnvGP and the postbinding events are early steps that are crucial to understand, as they could provide targets for the development of therapeutics. The better understanding of the envelope–receptor interaction also raises hopes for the possibility of designing entry machines that can deliver genes and other molecules to any cell of choice.

Recently, the use of whole genome siRNA screen has become more and more widely used for different viruses in order to identified factors important for entry. It should drastically increase our knowledge of the factors necessary for entry of viruses. Similarly, many high-throughput interactome studies will identify cellular proteins interacting with the different viral components. Altogether, the comparison of all these high-throughput screens should help us to identify cellular proteins and pathways common to different viruses which may help, with rational structure/mechanism-based design of entry inhibitors, to develop inhibitors that cross-react with different pathogens. In a similar fashion, the design of vaccine immunogens that are capable of eliciting potent, broadly neutralizing antibodies of known epitopes, is expected to contribute toward the development of vaccines. In parallel, the development of panels of human monoclonal antibodies against every entry-related protein from all pathogenic human viruses could accelerate our understanding of entry mechanisms and help to fight viral diseases. If research continues at the present pace, most of these goals could be accomplished within the next few decades.

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References

Aguilar, H. C., Anderson, W. F., and Cannon, P. M. (2003). Cytoplasmic tail of Moloney murine leukemia virus envelope protein influences the conformation of the extracellular domain: implications for mechanism of action of the R Peptide. *J. Virol.* **77,** 1281–1291.

Allison, A. C., and Valentine, R. C. (1960). Virus particle adsorption. II. Adsorption of vaccinia and fowl plague viruses to cells in suspension. *Biochim. Biophys. Acta* **40,** 393–399.

Alvarez, C. P., Lasala, F., Carrillo, J., Muniz, O., Corbi, A. L., and Delgado, R. (2002). C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. *J. Virol.* **76,** 6841–6844.

Anderson, M. M., Lauring, A. S., Burns, C. C., and Overbaugh, J. (2000). Identification of a cellular cofactor required for infection by feline leukemia virus. *Science* **287,** 1828–1830.

Andreadis, S., Lavery, T., Davis, H. E., Le Doux, J. M., Yarmush, M. L., and Morgan, J. R. (2000). Toward a more accurate quantitation of the activity of recombinant retroviruses: alternatives to titer and multiplicity of infection. *J. Virol.* **74,** 1258–1266.

Backovic, M., and Jardetzky, T. S. (2009). Class III viral membrane fusion proteins. *Curr. Opin. Struct. Bol.* **19,** 189–196.

Backovic, M., Longnecker, R., and Jardetzky, T. S. (2009). Class III viral membrane fusion proteins. *Proc. Natl. Acad. Sci. USA* **106,** 2880–2885.

Bae, Y., Kingsman, S. M., and Kingsman, A. J. (1997). Functional dissection of the Moloney murine leukemia virus envelope protein gp70. *J. Virol.* **71,** 2092–2099.

Bahnson, A. B., Dunigan, J. T., Baysal, B. E., Mohney, T., Atchison, R. W., Nimgaonkar, M. T., Ball, E. D., and Barranger, J. A. (1995). Centrifugal enhancement of retroviral mediated gene transfer. *J. Virol. Methods* **54,** 131–143.

Baldick, C. J., Wichroski, M. J., Pendri, A., Walsh, A. W., Fang, J., Mazzucco, C. E., Pokornowski, K. A., Rose, R. E., Eggers, B. J., Hsu, M., Zhai, W., Zhai, G., *et al.* (2010). A novel small molecule inhibitor of hepatitis C virus entry. *PLoS Pathog.* **6,** 100–110.

Balliet, J. W., and Bates, P. (1998). Efficient infection mediated by viral receptors incorporated into retroviral particles. *J. Virol.* **72,** 671–676.

Baranowski, E., Ruiz-Jarabo, C. M., and Domingo, E. (2001). Evolution of cell recognition by viruses. *Science* **292,** 1102–1105.

Barry, C., Key, T., Haddad, R., and Duncan, R. (2010). Features of a spatially constrained cystine loop in the p10 FAST protein ectodomain define a new class of viral fusion peptides. *J. Biol. Chem.* **285,** 16424–16433.

Bartosch, B., Dubuisson, J., and Cosset, F. L. (2003). Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. *J. Exp. Med.* **197,** 633–642.

Basak, A., Zhong, M., Munzer, J. S., Chretien, M., and Seidah, N. G. (2001). Implication of the proprotein convertases furin, PC5 and PC7 in the cleavage of surface glycoproteins of Hong Kong, Ebola and respiratory syncytial viruses: a comparative analysis with fluorogenic peptides. *Biochem. J.* **353,** 537–545.

Basak, A., Chretien, M., and Seidah, N. G. (2002). A rapid fluorometric assay for the proteolytic activity of SKI-1/S1P based on the surface glycoprotein of the hemorrhagic fever Lassa virus. *FEBS Lett.* **514,** 333–339.

Battini, J. L., Rasko, J. E., and Miller, A. D. (1999). A human cell-surface receptor for xenotropic and polytropic murine leukemia viruses: possible role in G protein-coupled signal transduction. *Proc. Natl. Acad. Sci. USA* **96,** 1385–1390.

Beavers, M. P., Myers, M. C., Shah, P. P., Purvis, J. E., Diamond, S. L., Cooperman, B. S., Huryn, D. M., and Smith, A. B., 3rd. (2008). Molecular docking of cathepsin L inhibitors in the binding site of papain. *J. Chem. Inf. Model.* **48,** 1464–1472.

Belouzard, S., Chu, V. C., and Whittaker, G. R. (2009). Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc. Natl. Acad. Sci. USA* **106,** 5871–5876.

Belouzard, S., Madu, I., and Whittaker, G. R. (2010). Elastase-mediated activation of the severe acute respiratory syndrome coronavirus spike protein at discrete sites within the S2 domain. *J. Biol. Chem.* **285,** 22758–22763.

Berger, K. L., Cooper, J. D., Heaton, N. S., Yoon, R., Oakland, T. E., Jordan, T. X., Mateu, G., Grakoui, A., and Randall, G. (2009). Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. *Proc. Natl. Acad. Sci. USA* **106,** 7577–7582.

Bergeron, E., Vincent, M. J., Wickham, L., Hamelin, J., Basak, A., Nichol, S. T., Chretien, M., and Seidah, N. G. (2005). Implication of proprotein convertases in the processing and spread of severe acute respiratory syndrome coronavirus. *Biochem. Biophys. Res. Commun.* **326,** 554–563.

Bertrand, P., Cote, M., Zheng, Y. M., Albritton, L. M., and Liu, S. L. (2008). Jaagsiekte sheep retrovirus utilizes a pH-dependent endocytosis pathway for entry. *J. Virol.* **82,** 2555–2559.

Beyer, W. R., Popplau, D., Garten, W., von Laer, D., and Lenz, O. (2003). Endoproteolytic processing of the lymphocytic choriomeningitis virus glycoprotein by the subtilase SKI-1/S1P. *J. Virol.* **77,** 2866–2872.

Bhattacharya, D., Logue, E. C., Bakkour, S., DeGregori, J., and Sha, W. C. (2002). Identification of gene function by cyclical packaging rescue of retroviral cDNA libraries. *Proc. Natl. Acad. Sci. USA* **99,** 8838–8843.

Bhattacharyya, S., Warfield, K. L., Ruthel, G., Bavari, S., Aman, M. J., and Hope, T. J. (2010). Ebola virus uses clathrin-mediated endocytosis as an entry pathway. *Virology* **401,** 18–28.

Blumenthal, R., Sarkar, D. P., Durell, S., Howard, D. E., and Morris, S. J. (1996). Dilation of the influenza hemagglutinin fusion pore revealed by the kinetics of individual cell-cell fusion events. *J. Cell Biol.* **135,** 63–71.

Bolken, T. C., Laquerre, S., Zhang, Y., Bailey, T. R., Pevear, D. C., Kickner, S. S., Sperzel, L. E., Jones, K. F., Warren, T. K., Amanda Lund, S., Kirkwood-Watts, D. L., King, D. S., *et al.* (2006). Identification and characterization of potent small molecule inhibitor of hemorrhagic fever New World arenaviruses. *Antiviral Res.* **69,** 86–97.

Borza, C. M., and Hutt-Fletcher, L. M. (2002). Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. *Nat. Med.* **8,** 594–599.

Boulay, F., Doms, R. W., Webster, R. G., and Helenius, A. (1988). Posttranslational oligomerization and cooperative acid activation of mixed influenza hemagglutinin trimers. *J. Cell Biol.* **106,** 629–639.

Bounou, S., Leclerc, J. E., and Tremblay, M. J. (2002). Presence of host ICAM-1 in laboratory and clinical strains of human immunodeficiency virus type 1 increases virus infectivity and CD4(+)-T-cell depletion in human lymphoid tissue, a major site of replication in vivo. *J. Virol.* **76,** 1004–1014.

Brass, A. L., Dykxhoorn, D. M., Benita, Y., Yan, N., Engelman, A., Xavier, R. J., Lieberman, J., and Elledge, S. J. (2008). Identification of host proteins required for HIV infection through a functional genomic screen. *Science* **319,** 921–926.

Broering, T. J., Garrity, K. A., Boatright, N. K., Sloan, S. E., Sandor, F., Thomas, W. D., Jr., Szabo, G., Finberg, R. W., Ambrosino, D. M., and Babcock, G. J. (2009). Identification and characterization of broadly neutralizing human monoclonal antibodies directed against the E2 envelope glycoprotein of hepatitis C virus. *J. Virol.* **83,** 12473–12482.

Buonocore, L., Blight, K. J., Rice, C. M., and Rose, J. K. (2002). Characterization of vesicular stomatitis virus recombinants that express and incorporate high levels of hepatitis C virus glycoproteins. *J. Virol.* **76,** 6865–6872.

Cantin, R., Fortin, J. F., Lamontagne, G., and Tremblay, M. (1997). The presence of host-derived HLA-DR1 on human immunodeficiency virus type 1 increases viral infectivity. *J. Virol.* **71,** 1922–1930.

Cantin, C., Holguera, J., Ferreira, L., Villar, E., and Munoz-Barroso, I. (2007). Newcastle disease virus may enter cells by caveolae-mediated endocytosis. *J. Gen. Virol.* **88,** 559–569.

Carneiro, F. A., Lapido-Loureiro, P. A., Cordo, S. M., Stauffer, F., Weissmuller, G., Bianconi, M. L., Juliano, M. A., Juliano, L., Bisch, P. M., and Da Poian, A. T. (2006). Probing the interaction between vesicular stomatitis virus and phosphatidylserine. *Eur. Biophys. J.* **35,** 145–154.

Catanese, M. T., Graziani, R., von Hahn, T., Moreau, M., Huby, T., Paonessa, G., Santini, C., Luzzago, A., Rice, C. M., Cortese, R., Vitelli, A., and Nicosia, A. (2007). High-avidity monoclonal antibodies against the human scavenger class B type I receptor efficiently block hepatitis C virus infection in the presence of high-density lipoprotein. *J. Virol.* **81,** 8063–8071.

Chakrabarti, L., Emerman, M., Tiollais, P., and Sonigo, P. (1989). The cytoplasmic domain of simian immunodeficiency virus transmembrane protein modulates infectivity. *J. Virol.* **63,** 4395–4403.

Chan, S. Y., Empig, C. J., Welte, F. J., Speck, R. F., Schmaljohn, A., Kreisberg, J. F., and Goldsmith, M. A. (2001). Folate receptor-alpha is a cofactor for cellular entry by Marburg and Ebola viruses. *Cell* **106,** 117–126.

Chandran, K., Sullivan, N. J., Felbor, U., Whelan, S. P., and Cunningham, J. M. (2005). Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. *Science* **308,** 1643–1645.

Cheng, G., Montero, A., Gastaminza, P., Whitten-Bauer, C., Wieland, S. F., Isogawa, M., Fredericksen, B., Selvarajah, S., Gallay, P. A., Ghadiri, M. R., and Chisari, F. V. (2008). A virocidal amphipathic {alpha}-helical peptide that inhibits hepatitis C virus infection in vitro. *Proc. Natl. Acad. Sci. USA* **105,** 3088–3093.

Chernomordik, L. V., and Kozlov, M. M. (2003). Protein-lipid interplay in fusion and fission of biological membranes. *Annu. Rev. Biochem.* **72,** 175–207.

Chernomordik, L. V., and Kozlov, M. M. (2005). Membrane hemifusion: crossing a chasm in two leaps. *Cell* **123,** 375–382.

Chernomordik, L. V., and Kozlov, M. M. (2008). Mechanics of membrane fusion. *Nat. Struct. Mol. Biol.* **15,** 675–683.

Cocchi, F., Fusco, D., Menotti, L., Gianni, T., Eisenberg, R. J., Cohen, G. H., and Campadelli-Fiume, G. (2004). The soluble ectodomain of herpes simplex virus gD contains a membrane-proximal pro-fusion domain and suffices to mediate virus entry. *Proc. Natl. Acad. Sci. USA* **101,** 7445–7450.

Coil, D. A., and Miller, A. D. (2004). Phosphatidylserine is not the cell surface receptor for vesicular stomatitis virus. *J. Virol.* **78,** 10920–10926.

Coil, D. A., and Miller, A. D. (2005). Enhancement of enveloped virus entry by phosphatidylserine. *J. Virol.* **79,** 11496–11500.

Coller, K. E., Berger, K. L., Heaton, N. S., Cooper, J. D., Yoon, R., and Randall, G. (2009). RNA interference and single particle tracking analysis of hepatitis C virus endocytosis. *PLoS Pathog.* **5,** e1000702.

Cormier, E. G., Tsamis, F., Kajumo, F., Durso, R. J., Gardner, J. P., and Dragic, T. (2004). CD81 is an entry coreceptor for hepatitis C virus. *Proc. Natl. Acad. Sci. USA* **101,** 7270–7274.

Cote, M., Zheng, Y. M., Albritton, L. M., and Liu, S. L. (2008). Fusogenicity of Jaagsiekte sheep retrovirus envelope protein is dependent on low pH and is enhanced by cytoplasmic tail truncations. *J. Virol.* **82,** 2543–2554.

Coyne, C. B., and Bergelson, J. M. (2006). Virus-induced Abl and Fyn kinase signals permit coxsackievirus entry through epithelial tight junctions. *Cell* **124,** 119–131.

Decroly, E., Wouters, S., Di Bello, C., Lazure, C., Ruysschaert, J. M., and Seidah, N. G. (1996). Identification of the paired basic convertases implicated in HIV gp160 processing based on in vitro assays and expression in CD4(+) cell lines. *J. Biol. Chem.* **271,** 30442–30450.

DeTulleo, L., and Kirchhausen, T. (1998). The clathrin endocytic pathway in viral infection. *EMBO J.* **17,** 4585–4593.

Diederich, S., Dietzel, E., and Maisner, A. (2009). Nipah virus fusion protein: influence of cleavage site mutations on the cleavability by cathepsin L, trypsin and furin. *Virus Res.* **145,** 300–306.

Drummer, H. E., and Poumbourios, P. (2004). Hepatitis C virus glycoprotein E2 contains a membrane-proximal heptad repeat sequence that is essential for E1E2 glycoprotein heterodimerization and viral entry. *J. Biol. Chem.* **279,** 30066–30072.

Drummer, H. E., Boo, I., and Poumbourios, P. (2007). Mutagenesis of a conserved fusion peptide-like motif and membrane-proximal heptad-repeat region of hepatitis C virus glycoprotein E1. *J. Virol.* **88,** 1144–1148.

Dube, D., Brecher, M. B., Delos, S. E., Rose, S. C., Park, E. W., Schornberg, K. L., Kuhn, J. H., and White, J. M. (2009). The primed ebolavirus glycoprotein (19-kilodalton GP1,2): sequence and residues critical for host cell binding. *J. Virol.* **83,** 2883–2891.

Duisit, G., Conrath, H., Saleun, S., Folliot, S., Provost, N., Cosset, F. L., Sandrin, V., Moullier, P., and Rolling, F. (2002). Five recombinant simian immunodeficiency virus pseudotypes lead to exclusive transduction of retinal pigmented epithelium in rat. *Mol. Ther.* **6,** 446–454.

Earp, L. J., Delos, S. E., Park, H. E., and White, J. M. (2005). The many mechanisms of viral membrane fusion proteins. *Curr. Top. Microbiol. Immunol.* **285,** 25–66.

Empig, C. J., and Goldsmith, M. A. (2002). Association of the caveola vesicular system with cellular entry by filoviruses. *J. Virol.* **76,** 5266–5270.

Endres, M. J., Jaffer, S., Haggarty, B., Turner, J. D., Doranz, B. J., O'Brien, P. J., Kolson, D. L., and Hoxie, J. A. (1997). Targeting of HIV- and SIV-infected cells by CD4-chemokine receptor pseudotypes. *Science* **278,** 1462–1464.

English, E. P., Chumanov, R. S., Gellman, S. H., and Compton, T. (2006). Rational development of beta-peptide inhibitors of human cytomegalovirus entry. *J. Biol. Chem.* **281,** 2661–2667.

Epand, R. M. (2003). Fusion peptides and the mechanism of viral fusion. *Biochim. Biophys. Acta* **1614,** 116–121.

Eren, R., Landstein, D., Terkieltaub, D., Nussbaum, O., Zauberman, A., Ben-Porath, J., Gopher, J., Buchnick, R., Kovjazin, R., Rosenthal-Galili, Z., Aviel, S., Ilan, E., *et al.* (2006). Preclinical evaluation of two neutralizing human monoclonal antibodies against hepatitis C virus (HCV): a potential treatment to prevent HCV reinfection in liver transplant patients. *J. Virol.* **80,** 2654–2664.

Ericsson, T. A., Takeuchi, Y., Templin, C., Quinn, G., Farhadian, S. F., Wood, J. C., Oldmixon, B. A., Suling, K. M., Ishii, J. K., Kitagawa, Y., Miyazawa, T., Salomon, D. R., *et al.* (2003). Identification of receptors for pig endogenous retrovirus. *Proc. Natl. Acad. Sci. USA* **100,** 6759–6764.

Evans, M. J., von Hahn, T., Tscherne, D. M., Syder, A. J., Panis, M., Wolk, B., Hatziioannou, T., McKeating, J. A., Bieniasz, P. D., and Rice, C. M. (2007). Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* **446,** 801–805.

Fenouillet, E., Barbouche, R., and Jones, I. M. (2007). Cell entry by enveloped viruses: redox considerations for HIV and SARS-coronavirus. *Antioxid. Redox Signal.* **9,** 1009–1034.

Fortin, J. F., Cantin, R., Lamontagne, G., and Tremblay, M. (1997). Host-derived ICAM-1 glycoproteins incorporated on human immunodeficiency virus type 1 are biologically active and enhance viral infectivity. *J. Virol.* **71,** 3588–3596.

Frampton, A. R., Jr., Stolz, D. B., Uchida, H., Goins, W. F., Cohen, J. B., and Glorioso, J. C. (2007). Equine herpesvirus 1 enters cells by two different pathways, and infection requires the activation of the cellular kinase ROCK1. *J. Virol.* **81,** 10879–10889.

Frecha, C., Costa, C., Levy, C., Negre, D., Russell, S. J., Maisner, A., Salles, G., Peng, K. W., Cosset, F. L., and Verhoeyen, E. (2009). Efficient and stable transduction of resting B lymphocytes and primary chronic lymphocyte leukemia cells using measles virus gp displaying lentiviral vectors. *Blood* **114,** 3173–3180.

Frecha, C., Costa, C., Negre, D., Gauthier, E., Russell, S. J., Cosset, F. L., and Verhoeyen, E. (2008a). Stable transduction of quiescent T cells without induction of cycle progression by a novel lentiviral vector pseudotyped with measles virus glycoproteins. *Blood* **112,** 4843–4852.

Frecha, C., Szecsi, J., Cosset, F. L., and Verhoeyen, E. (2008b). Strategies for targeting lentiviral vectors. *Curr. Gene Ther.* **8,** 449–460.

Gambaryan, A., Yamnikova, S., Lvov, D., Tuzikov, A., Chinarev, A., Pazynina, G., Webster, R., Matrosovich, M., and Bovin, N. (2005). Receptor specificity of influenza viruses from birds and mammals: new data on involvement of the inner fragments of the carbohydrate chain. *Virology* **334,** 276–283.

Garoff, H., Sjoberg, M., and Cheng, R. H. (2004). Budding of alphaviruses. *Virus Res.* **106,** 103–116.

Gastaminza, P., Whitten-Bauer, C., and Chisari, F. V. (2010). Unbiased probing of the entire hepatitis C virus life cycle identifies clinical compounds that target multiple aspects of the infection. *Proc. Natl. Acad. Sci. USA* **107,** 291–296.

Geijtenbeek, T. B., Kwon, D. S., Torensma, R., van Vliet, S. J., van Duijnhoven, G. C., Middel, J., Cornelissen, I. L., Nottet, H. S., KewalRamani, V. N., Littman, D. R., Figdor, C. G., and van Kooyk, Y. (2000). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* **100,** 587–597.

Giannecchini, S., Bonci, F., Pistello, M., Matteucci, D., Sichi, O., Rovero, P., and Bendinelli, M. (2004). The membrane-proximal tryptophan-rich region in the transmembrane glycoprotein ectodomain of feline immunodeficiency virus is important for cell entry. *Virology* **320,** 156–166.

Gianni, T., Forghieri, C., and Campadelli-Fiume, G. (2006). The herpesvirus glycoproteins B and H. L are sequentially recruited to the receptor-bound gD to effect membrane fusion at virus entry. *Proc. Natl. Acad. Sci. USA* **103,** 14572–14577.

Green, N., Shinnick, T. M., Witte, O., Ponticelli, A., Sutcliffe, J. G., and Lerner, R. A. (1981). Sequence-specific antibodies show that maturation of Moloney leukemia virus envelope polyprotein involves removal of a COOH-terminal peptide. *Proc. Natl. Acad. Sci. USA* **78,** 6023–6027.

Guibinga, G. H., Miyanohara, A., Esko, J. D., and Friedmann, T. (2002). Cell surface heparan sulfate is a receptor for attachment of envelope protein-free retrovirus-like particles and VSV-G pseudotyped MLV-derived retrovirus vectors to target cells. *Mol. Ther.* **5,** 538–546.

Habersetzer, F., Fournillier, A., Dubuisson, J., Rosa, D., Abrignani, S., Wychowski, C., Nakano, I., Trepo, C., Desgranges, C., and Inchauspe, G. (1998). Characterization of human monoclonal antibodies specific to the hepatitis C virus glycoprotein E2 with in vitro binding neutralization properties. *Virology* **249,** 32–41.

Hachiya, A., Sriwiriyanont, P., Patel, A., Saito, N., Ohuchi, A., Kitahara, T., Takema, Y., Tsuboi, R., Boissy, R. E., Visscher, M. O., Wilson, J. M., and Kobinger, G. P. (2007). Gene transfer in human skin with different pseudotyped HIV-based vectors. *Gene Ther.* **14,** 648–656.

Harrison, S. C. (2005). Mechanism of membrane fusion by viral envelope proteins. *Adv. Virus Res.* **64,** 231–261.

Harrison, S. C. (2008). Viral membrane fusion. *Nat. Struct. Mol. Biol.* **15,** 690–698.

Haywood, A. M. (1994). Virus receptors: binding, adhesion strengthening, and changes in viral structure. *J. Virol.* **68,** 1–5.

Heldwein, E. E., Lou, H., Bender, F. C., Cohen, G. H., Eisenberg, R. J., and Harrison, S. C. (2006). Crystal structure of glycoprotein B from herpes simplex virus 1. *Science* **313,** 217–220.

Helenius, A., Kartenbeck, J., Simons, K., and Fries, E. (1980). On the entry of Semliki forest virus into BHK-21 cells. *J. Cell Biol.* **84,** 404–420.

Helle, F., Wychowski, C., Vu-Dac, N., Gustafson, K. R., Voisset, C., and Dubuisson, J. (2006). Cyanovirin-N inhibits hepatitis C virus entry by binding to envelope protein glycans. *J. Biol. Chem.* **281,** 25177–25183.

Hood, C. L., Abraham, J., Boyington, J. C., Leung, K., Kwong, P. D., and Nabel, G. J. (2010). Biochemical and structural characterization of cathepsin L-processed Ebola virus glycoprotein: implications for viral entry and immunogenicity. *J. Virol.* **84,** 2972–2982.

Howard, M. W., Travanty, E. A., Jeffers, S. A., Smith, M. K., Wennier, S. T., Thackray, L. B., and Holmes, K. V. (2008). Aromatic amino acids in the juxtamembrane domain of severe acute respiratory syndrome coronavirus spike glycoprotein are important for receptor-dependent virus entry and cell-cell fusion. *J. Virol.* **82,** 2883–2894.

Hrobowski, Y. M., Garry, R. F., and Michael, S. F. (2005). Peptide inhibitors of dengue virus and West Nile virus infectivity. *Virol. J.* **2,** 49.

Hsu, M., Zhang, J., Flint, M., Logvinoff, C., Cheng-Mayer, C., Rice, C. M., and McKeating, J. A. (2003). Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. *Proc. Natl. Acad. Sci. USA* **100,** 7271–7276.

Huang, I. C., Bosch, B. J., Li, F., Li, W., Lee, K. H., Ghiran, S., Vasilieva, N., Dermody, T. S., Harrison, S. C., Dormitzer, P. R., Farzan, M., Rottier, P. J., *et al.* (2006). SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. *J. Biol. Chem.* **281,** 3198–3203.

Hunt, J. S., and Romanelli, F. (2009). Maraviroc, a CCR5 coreceptor antagonist that blocks entry of human immunodeficiency virus type 1. *Pharmacotherapy* **29,** 295–304.

Hunter, E. (1997). Viral entry and receptors. *In* "Retroviruses" (J. Coffin, S. Hughes, and H. Varmus, eds.), pp. 71–120. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Jeetendra, E., Robison, C. S., Albritton, L. M., and Whitt, M. A. (2002). The membrane-proximal domain of vesicular stomatitis virus G protein functions as a membrane fusion potentiator and can induce hemifusion. *J. Virol.* **76,** 12300–12311.

Jeetendra, E., Ghosh, K., Odell, D., Li, J., Ghosh, H. P., and Whitt, M. A. (2003). The membrane-proximal region of vesicular stomatitis virus glycoprotein G ectodomain is critical for fusion and virus infectivity. *J. Virol.* **77,** 12807–12818.

Ji, C., Zhang, J., Dioszegi, M., Chiu, S., Rao, E., Derosier, A., Cammack, N., Brandt, M., and Sankuratri, S. (2007). CCR5 small-molecule antagonists and monoclonal antibodies exert potent synergistic antiviral effects by cobinding to the receptor. *Mol. Pharmacol.* **72,** 18–28.

Jin, M., Park, J., Lee, S., Park, B., Shin, J., Song, K. J., Ahn, T. I., Hwang, S. Y., Ahn, B. Y., and Ahn, K. (2002). Hantaan virus enters cells by clathrin-dependent receptor-mediated endocytosis. *Virology* **294,** 60–69.

Jinno-Oue, A., Oue, M., and Ruscetti, S. K. (2001). A unique heparin-binding domain in the envelope protein of the neuropathogenic PVC-211 murine leukemia virus may contribute to its brain capillary endothelial cell tropism. *J. Virol.* **75,** 12439–12445.

Joshi, S. B., Dutch, R. E., and Lamb, R. A. (1998). A core trimer of the paramyxovirus fusion protein: parallels to influenza virus hemagglutinin and HIV-1 gp41. *Virology* **248,** 20–34.

Kabat, D., Kozak, S. L., Wehrly, K., and Chesebro, B. (1994). Differences in CD4 dependence for infectivity of laboratory-adapted and primary patient isolates of human immunodeficiency virus type 1. *J. Virol.* **68,** 2570–2577.

Kadlec, J., Loureiro, S., Abrescia, N. G., Stuart, D. I., and Jones, I. M. (2008). The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines. *Nat. Struct. Mol. Biol.* **15,** 1024–1030.

Kaletsky, R. L., Simmons, G., and Bates, P. (2007). Proteolysis of the Ebola virus glycoproteins enhances virus binding and infectivity. *J. Virol.* **81,** 13378–13384.

Kang, Y., Stein, C. S., Heth, J. A., Sinn, P. L., Penisten, A. K., Staber, P. D., Ratliff, K. L., Shen, H., Barker, C. K., Martins, I., Sharkey, C. M., Sanders, D. A., *et al.* (2002). In vivo gene transfer using a nonprimate lentiviral vector pseudotyped with Ross River Virus glycoproteins. *J. Virol.* **76,** 9378–9388.

Kang, Y., Xie, L., Tran, D. T., Stein, C. S., Hickey, M., Davidson, B. L., and McCray, P. B., Jr. (2005). Persistent expression of factor VIII in vivo following nonprimate lentiviral gene transfer. *Blood* **106,** 1552–1558.

Karlas, A., Machuy, N., Shin, Y., Pleissner, K. P., Artarini, A., Heuer, D., Becker, D., Khalil, H., Ogilvie, L. A., Hess, S., Maurer, A. P., Muller, E., *et al.* (2010). Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature* **463,** 818–822.

Kaushik-Basu, N., Basu, A., and Harris, D. (2008). Peptide inhibition of HIV-1: current status and future potential. *BioDrugs* **22,** 161–175.

Keck, Z. Y., Machida, K., Lai, M. M., Ball, J. K., Patel, A. H., and Foung, S. K. (2008). Therapeutic control of hepatitis C virus: the role of neutralizing monoclonal antibodies. *Curr. Top. Microbiol. Immunol.* **317,** 1–38.

Kee, S. H., Cho, E. J., Song, J. W., Park, K. S., Baek, L. J., and Song, K. J. (2004). Effects of endocytosis inhibitory drugs on rubella virus entry into VeroE6 cells. *Microbiol. Immunol.* **48,** 823–829.

Kemble, G. W., Danieli, T., and White, J. M. (1994). Lipid-anchored influenza hemagglutinin promotes hemifusion, not complete fusion. *Cell* **76,** 383–391.

Kielian, M., and Rey, F. A. (2006). Virus membrane-fusion proteins: more than one way to make a hairpin. *Nat. Rev. Microbiol.* **4,** 67–76.

Kobinger, G. P., Weiner, D. J., Yu, Q. C., and Wilson, J. M. (2001). Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia in vivo. *Nat. Biotechnol.* **19,** 225–230.

Koh, E. Y., Chen, T., and Daley, G. Q. (2002). Novel retroviral vectors to facilitate expression screens in mammalian cells. *Nucleic Acids Res.* **30,** e142.

Kolokoltsov, A. A., Deniger, D., Fleming, E. H., Roberts, N. J., Jr., Karpilow, J. M., and Davey, R. A. (2007). Small interfering RNA profiling reveals key role of clathrin-mediated endocytosis and early endosome formation for infection by respiratory syncytial virus. *J. Virol.* **81,** 7786–7800.

Konig, R., Zhou, Y., Elleder, D., Diamond, T. L., Bonamy, G. M., Irelan, J. T., Chiang, C. Y., Tu, B. P., De Jesus, P. D., Lilley, C. E., Seidel, S., Opaluch, A. M., *et al.* (2008). Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. *Cell* **135,** 49–60.

Koutsoudakis, G., Kaul, A., Steinmann, E., Kallis, S., Lohmann, V., Pietschmann, T., and Bartenschlager, R. (2006). Characterization of the early steps of hepatitis C virus infection by using luciferase reporter viruses. *J. Virol.* **80,** 5308–5320.

Kowalski, M., Potz, J., Basiripour, L., Dorfman, T., Goh, W. C., Terwilliger, E., Dayton, A., Rosen, C., Haseltine, W., and Sodroski, J. (1987). Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. *Science* **237,** 1351–1355.

Kowolik, C. M., and Yee, J. K. (2002). Preferential transduction of human hepatocytes with lentiviral vectors pseudotyped by Sendai virus F protein. *Mol. Ther.* **5,** 762–769.

Krieger, S. E., Zeisel, M. B., Davis, C., Thumann, C., Harris, H. J., Schnober, E. K., Mee, C., Soulier, E., Royer, C., Lambotin, M., Grunert, F., Dao Thi, V. L., *et al.* (2010). Inhibition of hepatitis C virus infection by anti-claudin-1 antibodies is mediated by neutralization of E2-CD81-claudin-1 associations. *Hepatology* **51,** 1144–1157.

Krishnan, M. N., Ng, A., Sukumaran, B., Gilfoy, F. D., Uchil, P. D., Sultana, H., Brass, A. L., Adametz, R., Tsui, M., Qian, F., Montgomery, R. R., Lev, S., *et al.* (2008). RNA interference screen for human genes associated with West Nile virus infection. *Nature* **455,** 242–245.

Kuhmann, S. E., Platt, E. J., Kozak, S. L., and Kabat, D. (2000). Cooperation of multiple CCR5 coreceptors is required for infections by human immunodeficiency virus type 1. *J. Virol.* **74,** 7005–7015.

Kuhn, R. J., Zhang, W., Rossmann, M. G., Pletnev, S. V., Corver, J., Lenches, E., Jones, C. T., Mukhopadhyay, S., Chipman, P. R., Strauss, E. G., Baker, T. S., and Strauss, J. H. (2002). Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell* **108,** 717–725.

Kwon, H., Bai, Q., Baek, H. J., Felmet, K., Burton, E. A., Goins, W. F., Cohen, J. B., and Glorioso, J. C. (2006). Soluble V domain of Nectin-1/HveC enables entry of herpes simplex virus type 1 (HSV-1) into HSV-resistant cells by binding to viral glycoprotein D. *J. Virol.* **80,** 138–148.

Labonte, P., Begley, S., Guevin, C., Asselin, M. C., Nassoury, N., Mayer, G., Prat, A., and Seidah, N. G. (2009). PCSK9 impedes hepatitis C virus infection in vitro and modulates liver CD81 expression. *Hepatology* **50,** 17–24.

Labonte, P., and Seidah, N. G. (2008). Emerging viruses: risk of pandemic. *Expert Rev Anti Infect Ther* **6,** 581–583.

Lahlil, R., Calvo, F., and Khatib, A. M. (2009). The potential anti-tumorigenic and anti-metastatic side of the proprotein convertases inhibitors. *Recent Pat. Anticancer Drug Discov.* **4,** 83–91.

Lambert, D. M., Barney, S., Lambert, A. L., Guthrie, K., Medinas, R., Davis, D. E., Bucy, T., Erickson, J., Merutka, G., and Petteway, S. R., Jr. (1996). Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion. *Proc. Natl. Acad. Sci. USA* **93,** 2186–2191.

Larson, R. A., Dai, D., Hosack, V. T., Tan, Y., Bolken, T. C., Hruby, D. E., and Amberg, S. M. (2008). Identification of a broad-spectrum arenavirus entry inhibitor. *J. Virol.* **82,** 10768–10775.

Lavillette, D., Maurice, M., Roche, C., Russell, S. J., Sitbon, M., and Cosset, F. L. (1998). A proline-rich motif downstream of the receptor binding domain modulates conformation and fusogenicity of murine retroviral envelopes. *J. Virol.* **72,** 9955–9965.

Lavillette, D., Ruggieri, A., Russell, S. J., and Cosset, F. L. (2000). Activation of a cell entry pathway common to type C mammalian retroviruses by soluble envelope fragments. *J. Virol.* **74,** 295–304.

Lavillette, D., Marin, M., Ruggieri, A., Mallet, F., Cosset, F. L., and Kabat, D. (2002). The envelope glycoprotein of human endogenous retrovirus type W uses a divergent family of amino acid transporters/cell surface receptors. *J. Virol.* **76,** 6442–6452.

Lavillette, D., Tarr, A. W., Voisset, C., Donot, P., Bartosch, B., Bain, C., Patel, A. H., Dubuisson, J., Ball, J. K., and Cosset, F. L. (2005). Characterization of host-range and cell entry properties of the major genotypes and subtypes of hepatitis C virus. *Hepatology* **41,** 265–274.

Law, M., Maruyama, T., Lewis, J., Giang, E., Tarr, A. W., Stamataki, Z., Gastaminza, P., Chisari, F. V., Jones, I. M., Fox, R. I., Ball, J. K., McKeating, J. A., *et al.* (2008). Broadly neutralizing antibodies protect against hepatitis C virus quasispecies challenge. *Nat. Med.* **14,** 25–27.

Lee, J. E., Fusco, M. L., Hessell, A. J., Oswald, W. B., Burton, D. R., and Saphire, E. O. (2008). Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. *Nature* **454,** 177–182.

Lenz, O., ter Meulen, J., Klenk, H. D., Seidah, N. G., and Garten, W. (2001). The Lassa virus glycoprotein precursor GP-C is proteolytically processed by subtilase SKI-1/S1P. *Proc. Natl. Acad. Sci. USA* **98,** 12701–12705.

Leonard, J. T., and Roy, K. (2006). The HIV entry inhibitors revisited. *Curr. Med. Chem.* **13,** 911–934.

Lescar, J., Roussel, A., Wien, M. W., Navaza, J., Fuller, S. D., Wengler, G., and Rey, F. A. (2001). The Fusion glycoprotein shell of Semliki Forest virus: an icosahedral assembly primed for fusogenic activation at endosomal pH. *Cell* **105,** 137–148.

Li, M., Yang, C., Tong, S., Weidmann, A., and Compans, R. W. (2002). Palmitoylation of the murine leukemia virus envelope protein is critical for lipid raft association and surface expression. *J. Virol.* **76,** 11845–11852.

Li, K., Zhang, S., Kronqvist, M., Wallin, M., Ekstrom, M., Derse, D., and Garoff, H. (2008). Intersubunit disulfide isomerization controls membrane fusion of human T-cell leukemia virus Env. *J. Virol.* **82,** 7135–7143.

Li, Q., Brass, A. L., Ng, A., Hu, Z., Xavier, R. J., Liang, T. J., and Elledge, S. J. (2009). A genome-wide genetic screen for host factors required for hepatitis C virus propagation. *Proc. Natl. Acad. Sci. USA* **106,** 16410–16415.

Liu, S., Lu, H., Niu, J., Xu, Y., Wu, S., and Jiang, S. (2005). Different from the HIV fusion inhibitor C34, the anti-HIV drug Fuzeon (T-20) inhibits HIV-1 entry by targeting multiple sites in gp41 and gp120. *J. Biol. Chem.* **280,** 11259–11273.

Liu, S., Wu, S., and Jiang, S. (2007). HIV entry inhibitors targeting gp41: from polypeptides to small-molecule compounds. *Curr. Pharm. Des.* **13,** 143–162.

Lopper, M., and Compton, T. (2004). Coiled-coil domains in glycoproteins B and H are involved in human cytomegalovirus membrane fusion. *J. Virol.* **78,** 8333–8341.

Lorizate, M., Cruz, A., Huarte, N., Kunert, R., Perez-Gil, J., and Nieva, J. L. (2006). Recognition and blocking of HIV-1 gp41 pre-transmembrane sequence by monoclonal 4E10 antibody in a Raft-like membrane environment. *J. Biol. Chem.* **281,** 39598–39606.

Loving, R., Li, K., Wallin, M., Sjoberg, M., and Garoff, H. (2008). R-Peptide cleavage potentiates fusion-controlling isomerization of the intersubunit disulfide in Moloney murine leukemia virus Env. *J. Virol.* **82,** 2594–2597.

Madshus, I. H., Sandvig, K., Olsnes, S., and van Deurs, B. (1987). Effect of reduced endocytosis induced by hypotonic shock and potassium depletion on the infection of Hep 2 cells by picorna-viruses. *J. Cell. Physiol.* **131,** 14–22.

Marechal, V., Prevost, M. C., Petit, C., Perret, E., Heard, J. M., and Schwartz, O. (2001). Human immunodeficiency virus type 1 entry into macrophages mediated by macropinocytosis. *J. Virol.* **75,** 11166–11177.

Marin, M., Tailor, C. S., Nouri, A., Kozak, S. L., and Kabat, D. (1999). Polymorphisms of the cell surface receptor control mouse susceptibilities to xenotropic and polytropic leukemia viruses. *J. Virol.* **73,** 9362–9368.

Markosyan, R. M., Cohen, F. S., and Melikyan, G. B. (2000). The lipid-anchored ectodomain of influenza virus hemagglutinin (GPI-HA) is capable of inducing nonenlarging fusion pores. *Mol. Biol. Cell* **11,** 1143–1152.

Marsh, M., and Helenius, A. (2006). Virus entry: open sesame. *Cell* **124,** 729–740.

Marzi, A., Akhavan, A., Simmons, G., Gramberg, T., Hofmann, H., Bates, P., Lingappa, V. R., and Pohlmann, S. (2006). The signal peptide of the ebolavirus glycoprotein influences interaction with the cellular lectins DC-SIGN and DC-SIGNR. *J. Virol.* **80,** 6305–6317.

Mason, P. W., Rieder, E., and Baxt, B. (1994). RGD sequence of foot-and-mouth disease virus is essential for infecting cells via the natural receptor but can be bypassed by an antibody-dependent enhancement pathway. *Proc. Natl. Acad. Sci. USA* **91,** 1932–1936.

Matlin, K. S., Reggio, H., Helenius, A., and Simons, K. (1981). Infectious entry pathway of influenza virus in a canine kidney cell line. *J. Cell Biol.* **91,** 601–613.

Matsuno, K., Kishida, N., Usami, K., Igarashi, M., Yoshida, R., Nakayama, E., Shimojima, M., Feldmann, H., Irimura, T., Kawaoka, Y., and Takada, A. (2010). Different potential of C-type lectin-mediated entry between Marburg virus strains. *J. Virol.* **84,** 5140–5147.

Matsuyama, S., and Taguchi, F. (2002). Receptor-induced conformational changes of murine coronavirus spike protein. *J. Virol.* **76,** 11819–11826.

Matsuyama, S., Ujike, M., Morikawa, S., Tashiro, M., and Taguchi, F. (2005). Protease-mediated enhancement of severe acute respiratory syndrome coronavirus infection. *Proc. Natl. Acad. Sci. USA* **102,** 12543–12547.

Mayor, S., and Pagano, R. E. (2007). Pathways of clathrin-independent endocytosis. *Nat. Rev. Mol. Cell Biol.* **8,** 603–612.

Mebatsion, T., Finke, S., Weiland, F., and Conzelmann, K. K. (1997). A CXCR4/CD4 pseudotype rhabdovirus that selectively infects HIV-1 envelope protein-expressing cells. *Cell* **90,** 841–847.

Melikyan, G. B. (2008). Common principles and intermediates of viral protein-mediated fusion: the HIV-1 paradigm. *Retrovirology* **5,** 111.

Melikyan, G. B., Barnard, R. J., Abrahamyan, L. G., Mothes, W., and Young, J. A. (2005). Imaging individual retroviral fusion events: from hemifusion to pore formation and growth. *Proc. Natl. Acad. Sci. USA* **102,** 8728–8733.

Mercer, J., and Helenius, A. (2008). Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. *Science* **320,** 531–535.

Mercer, J., and Helenius, A. (2009). Virus entry by macropinocytosis. *Nat. Cell Biol.* **11,** 510–520.

Mercer, J., Knebel, S., Schmidt, F. I., Crouse, J., Burkard, C., and Helenius, A. (2010a). Vaccinia virus strains use distinct forms of macropinocytosis for host-cell entry. *Proc. Natl. Acad. Sci. USA* **107,** 9346–9351.

Mercer, J., Schelhaas, M., and Helenius, A. (2010b). Virus entry by endocytosis. *Annu. Rev. Biochem.* **79,** 803–833.

Meuleman, P., Hesselgesser, J., Paulson, M., Vanwolleghem, T., Desombere, I., Reiser, H., and Leroux-Roels, G. (2008). Anti-CD81 antibodies can prevent a hepatitis C virus infection in vivo. *Hepatology* **48,** 1761–1768.

Miletic, H., Fischer, Y. H., Neumann, H., Hans, V., Stenzel, W., Giroglou, T., Hermann, M., Deckert, M., and Von Laer, D. (2004). Selective transduction of malignant glioma by lentiviral vectors pseudotyped with lymphocytic choriomeningitis virus glycoproteins. *Hum. Gene Ther.* **15,** 1091–1100.

Miller, N., and Hutt-Fletcher, L. M. (1992). Epstein-Barr virus enters B cells and epithelial cells by different routes. *J. Virol.* **66,** 3409–3414.

Molina, S., Castet, V., Pichard-Garcia, L., Wychowski, C., Meurs, E., Pascussi, J. M., Sureau, C., Fabre, J. M., Sacunha, A., Larrey, D., Dubuisson, J., Coste, J., *et al.* (2008). Serum-derived hepatitis C virus infection of primary human hepatocytes is tetraspanin CD81 dependent. *J. Virol.* **82,** 569–574.

Moll, M., Klenk, H. D., and Maisner, A. (2002). Importance of the cytoplasmic tails of the measles virus glycoproteins for fusogenic activity and the generation of recombinant measles viruses. *J. Virol.* **76,** 7174–7186.

Mondor, I., Ugolini, S., and Sattentau, Q. J. (1998). Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans. *J. Virol.* **72,** 3623–3634.

Moreno, M. R., Giudici, M., and Villalain, J. (2006). The membranotropic regions of the endo and ecto domains of HIV gp41 envelope glycoprotein. *Biochim. Biophys. Acta* **1758,** 111–123.

Moser, T. S., Jones, R. G., Thompson, C. B., Coyne, C. B., and Cherry, S. (2010). A kinome RNAi screen identified AMPK as promoting poxvirus entry through the control of actin dynamics. *PLoS Pathog.* **6,** e1000954.

Mothes, W., Boerger, A. L., Narayan, S., Cunningham, J. M., and Young, J. A. (2000). Retroviral entry mediated by receptor priming and low pH triggering of an envelope glycoprotein. *Cell* **103,** 679–689.

Mulligan, M. J., Yamshchikov, G. V., Ritter, G. D., Jr., Gao, F., Jin, M. J., Nail, C. D., Spies, C. P., Hahn, B. H., and Compans, R. W. (1992). Cytoplasmic domain truncation enhances fusion activity by the exterior glycoprotein complex of human immunodeficiency virus type 2 in selected cell types. *J. Virol.* **66,** 3971–3975.

Munoz-Barroso, I., Durell, S., Sakaguchi, K., Appella, E., and Blumenthal, R. (1998). Dilation of the human immunodeficiency virus-1 envelope glycoprotein fusion pore revealed by the inhibitory action of a synthetic peptide from gp41. *J. Cell Biol.* **140,** 315–323.

Munoz-Barroso, I., Salzwedel, K., Hunter, E., and Blumenthal, R. (1999). Role of the membrane-proximal domain in the initial stages of human immunodeficiency virus type 1 envelope glycoprotein-mediated membrane fusion. *J. Virol.* **73,** 6089–6092.

Murga, J. D., Franti, M., Pevear, D. C., Maddon, P. J., and Olson, W. C. (2006). Potent antiviral synergy between monoclonal antibody and small-molecule CCR5 inhibitors of human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **50,** 3289–3296.

Myers, M. C., Shah, P. P., Beavers, M. P., Napper, A. D., Diamond, S. L., Smith, A. B., 3rd, and Huryn, D. M. (2008). Design, synthesis, and evaluation of inhibitors of cathepsin L: Exploiting a unique thiocarbazate chemotype. *Bioorg. Med. Chem. Lett.* **18,** 3646–3651.

Ng, T. I., Mo, H., Pilot-Matias, T., He, Y., Koev, G., Krishnan, P., Mondal, R., Pithawalla, R., He, W., Dekhtyar, T., Packer, J., Schurdak, M., *et al.* (2007). Identification of host genes involved in hepatitis C virus replication by small interfering RNA technology. *Hepatology* **45,** 1413–1421.

Ochsenbauer-Jambor, C., Miller, D. C., Roberts, C. R., Rhee, S. S., and Hunter, E. (2001). Palmitoylation of the Rous sarcoma virus transmembrane glycoprotein is required for protein stability and virus infectivity. *J. Virol.* **75,** 11544–11554.

Okumura, Y., Takahashi, E., Yano, M., Ohuchi, M., Daidoji, T., Nakaya, T., Bottcher, E., Garten, W., Klenk, H. D., and Kido, H. (2010). Novel type II transmembrane serine proteases, MSPL and TMPRSS13, Proteolytically activate membrane fusion activity of the hemagglutinin of highly pathogenic avian influenza viruses and induce their multicycle replication. *J. Virol.* **84,** 5089–5096.

Oldstone, M. B., Lewicki, H., Thomas, D., Tishon, A., Dales, S., Patterson, J., Manchester, M., Homann, D., Naniche, D., and Holz, A. (1999). Measles virus infection in a transgenic model: virus-induced immunosuppression and central nervous system disease. *Cell* **98,** 629–640.

Oomens, A. G., Bevis, K. P., and Wertz, G. W. (2006). The cytoplasmic tail of the human respiratory syncytial virus F protein plays critical roles in cellular localization of the F protein and infectious progeny production. *J. Virol.* **80,** 10465–10477.

Owens, R. J., Burke, C., and Rose, J. K. (1994). Mutations in the membrane-spanning domain of the human immunodeficiency virus envelope glycoprotein that affect fusion activity. *J. Virol.* **68,** 570–574.

Owsianka, A., Tarr, A. W., Juttla, V. S., Lavillette, D., Bartosch, B., Cosset, F. L., Ball, J. K., and Patel, A. H. (2005). Monoclonal antibody AP33 defines a broadly neutralizing epitope on the hepatitis C virus E2 envelope glycoprotein. *J. Virol.* **79,** 11095–11104.

Ozden, S., Lucas-Hourani, M., Ceccaldi, P. E., Basak, A., Valentine, M., Benjannet, S., Hamelin, J., Jacob, Y., Mamchaoui, K., Mouly, V., Despres, P., Gessain, A., *et al.* (2008). Inhibition of Chikungunya virus infection in cultured human muscle cells by furin inhibitors: impairment of the maturation of the E2 surface glycoprotein. *J. Biol. Chem.* **283,** 21899–21908.

Pager, C. T., and Dutch, R. E. (2005). Cathepsin L is involved in proteolytic processing of the Hendra virus fusion protein. *J. Virol.* **79,** 12714–12720.

Palermo, C., and Joyce, J. A. (2008). Cysteine cathepsin proteases as pharmacological targets in cancer. *Trends Pharmacol. Sci.* **29,** 22–28.

Park, P. W., Reizes, O., and Bernfield, M. (2000). Cell surface heparan sulfate proteoglycans: selective regulators of ligand-receptor encounters. *J. Biol. Chem.* **275,** 29923–29926.

Parker, J. S., Murphy, W. J., Wang, D., O'Brien, S. J., and Parrish, C. R. (2001). Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter, and infect cells. *J. Virol.* **75,** 3896–3902.

Pecheur, E. I., Lavillette, D., Alcaras, F., Molle, J., Boriskin, Y. S., Roberts, M., Cosset, F. L., and Polyak, S. J. (2007). Biochemical mechanism of hepatitis C virus inhibition by the broad-spectrum antiviral arbidol. *Biochemistry* **46,** 6050–6059.

Pernet, O., Pohl, C., Ainouze, M., Kweder, H., and Buckland, R. (2009). Nipah virus entry can occur by macropinocytosis. *Virology* **395,** 298–311.

Perotti, M., Mancini, N., Diotti, R. A., Tarr, A. W., Ball, J. K., Owsianka, A., Adair, R., Patel, A. H., Clementi, M., and Burioni, R. (2008). Identification of a broadly cross-reacting and neutralizing human monoclonal antibody directed against the hepatitis C virus E2 protein. *J. Virol.* **82,** 1047–1052.

Pinter, A., Kopelman, R., Li, Z., Kayman, S. C., and Sanders, D. A. (1997). Localization of the labile disulfide bond between SU and TM of the murine leukemia virus envelope protein complex to a highly conserved CWLC motif in SU that resembles the active-site sequence of thiol-disulfide exchange enzymes. *J. Virol.* **71,** 8073–8077.

Pizzato, M., Marlow, S. A., Blair, E. D., and Takeuchi, Y. (1999). Initial binding of murine leukemia virus particles to cells does not require specific Env-receptor interaction. *J. Virol.* **73,** 8599–8611.

Pizzato, M., Blair, E. D., Fling, M., Kopf, J., Tomassetti, A., Weiss, R. A., and Takeuchi, Y. (2001). Evidence for nonspecific adsorption of targeted retrovirus vector particles to cells.. *Gene Ther.* **8,** 1088–1096.

Pizzato, M., Popova, E., and Gottlinger, H. G. (2008). Nef can enhance the infectivity of receptor-pseudotyped human immunodeficiency virus type 1 particles. *J. Virol.* **82,** 10811–10819.

Platt, E. J., Wehrly, K., Kuhmann, S. E., Chesebro, B., and Kabat, D. (1998). Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1. *J. Virol.* **72,** 2855–2864.

Ploss, A., and Rice, C. M. (2009). Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *EMBO Rep.* **10,** 1220–1227.

Ploss, A., Evans, M. J., Gaysinskaya, V. A., Panis, M., You, H., de Jong, Y. P., and Rice, C. M. (2009). Towards a small animal model for hepatitis C. *Nature* **457,** 882–886.

Pohlmann, S., Soilleux, E. J., Baribaud, F., Leslie, G. J., Morris, L. S., Trowsdale, J., Lee, B., Coleman, N., and Doms, R. W. (2001). DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. *Proc. Natl. Acad. Sci. USA* **98,** 2670–2675.

Porotto, M., Yokoyama, C. C., Palermo, L. M., Mungall, B., Aljofan, M., Cortese, R., Pessi, A., and Moscona, A. (2010). Viral entry inhibitors targeted to the membrane site of action. *J. Virol.* **84,** 6760–6768.

Pullikotil, P., Vincent, M., Nichol, S. T., and Seidah, N. G. (2004). Development of protein-based inhibitors of the proprotein of convertase SKI-1/S1P: processing of SREBP-2, ATF6, and a viral glycoprotein. *J. Biol. Chem.* **279,** 17338–17347.

Purtscher, M., Trkola, A., Gruber, G., Buchacher, A., Predl, R., Steindl, F., Tauer, C., Berger, R., Barrett, N., Jungbauer, A., *et al.* (1994). A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1. *AIDS Res. Hum. Retroviruses* **10,** 1651–1658.

Qiu, Z., Hingley, S. T., Simmons, G., Yu, C., Das Sarma, J., Bates, P., and Weiss, S. R. (2006). Endosomal proteolysis by cathepsins is necessary for murine coronavirus mouse hepatitis virus type 2 spike-mediated entry. *J. Virol.* **80,** 5768–5776.

Quigley, J. G., Burns, C. C., Anderson, M. M., Lynch, E. D., Sabo, K. M., Overbaugh, J., and Abkowitz, J. L. (2000). Cloning of the cellular receptor for feline leukemia virus subgroup C (FeLV-C), a retrovirus that induces red cell aplasia. *Blood* **95,** 1093–1099.

Quinn, K., Brindley, M. A., Weller, M. L., Kaludov, N., Kondratowicz, A., Hunt, C. L., Sinn, P. L., McCray, P. B., Jr., Stein, C. S., Davidson, B. L., Flick, R., Mandell, R., *et al.* (2009). Rho GTPases modulate entry of Ebola virus and vesicular stomatitis virus pseudotyped vectors. *J. Virol.* **83,** 10176–10186.

Rapaport, D., Ovadia, M., and Shai, Y. (1995). A synthetic peptide corresponding to a conserved heptad repeat domain is a potent inhibitor of Sendai virus-cell fusion: an emerging similarity with functional domains of other viruses. *EMBO J.* **14,** 5524–5531.

Rasko, J. E., Battini, J. L., Gottschalk, R. J., Mazo, I., and Miller, A. D. (1999). The RD114/simian type D retrovirus receptor is a neutral amino acid transporter. *Proc. Natl. Acad. Sci. USA* **96,** 2129–2134.

Rein, A., Mirro, J., Haynes, J. G., Ernst, S. M., and Nagashima, K. (1994). Function of the cytoplasmic domain of a retroviral transmembrane protein: p15E-p2E cleavage activates the membrane fusion capability of the murine leukemia virus env protein. *J. Virol.* **68,** 1773–1781.

Rey, F. A. (2006). Molecular gymnastics at the herpesvirus surface. *EMBO Rep.* **7,** 1000–1005.
Rey, F. A., Heinz, F. X., Mandl, C., Kunz, C., and Harrison, S. C. (1995). The envelope glycoprotein from tick-borne encephalitis virus at 2 A resolution. *Nature* **375,** 291–298.
Roche, S., and Gaudin, Y. (2002). Characterization of the equilibrium between the native and fusion-inactive conformation of rabies virus glycoprotein indicates that the fusion complex is made of several trimers. *Virology* **297,** 128–135.
Roche, S., Bressanelli, S., Rey, F. A., and Gaudin, Y. (2006). Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein G. *Science* **313,** 187–191.
Roche, S., Rey, F. A., Gaudin, Y., and Bressanelli, S. (2007). Structure of the prefusion form of the vesicular stomatitis virus glycoprotein G. *Science* **315,** 843–848.
Roche, S., Albertini, A. A., Lepault, J., Bressanelli, S., and Gaudin, Y. (2008). Structures of vesicular stomatitis virus glycoprotein: membrane fusion revisited. *Cell. Mol. Life Sci.* **65,** 1716–1728.
Root, M. J., Kay, M. S., and Kim, P. S. (2001). Protein design of an HIV-1 entry inhibitor. *Science* **291,** 884–888.
Rossi, A., Deveraux, Q., Turk, B., and Sali, A. (2004). Comprehensive search for cysteine cathepsins in the human genome. *Biol. Chem.* **385,** 363–372.
Rozenberg-Adler, Y., Conner, J., Aguilar-Carreno, H., Chakraborti, S., Dimitrov, D. S., and Anderson, W. F. (2008). Membrane-proximal cytoplasmic domain of Moloney murine leukemia virus envelope tail facilitates fusion. *Exp. Mol. Pathol.* **84,** 18–30.
Saez-Cirion, A., Gomara, M. J., Agirre, A., and Nieva, J. L. (2003). Pre-transmembrane sequence of Ebola glycoprotein. Interfacial hydrophobicity distribution and interaction with membranes. *FEBS Lett.* **533,** 47–53.
Salminen, A., Wahlberg, J. M., Lobigs, M., Liljestrom, P., and Garoff, H. (1992). Membrane fusion process of Semliki Forest virus. II: Cleavage-dependent reorganization of the spike protein complex controls virus entry. *J. Cell Biol.* **116,** 349–357.
Salzwedel, K., West, J. T., and Hunter, E. (1999). A conserved tryptophan-rich motif in the membrane-proximal region of the human immunodeficiency virus type 1 gp41 ectodomain is important for Env-mediated fusion and virus infectivity. *J. Virol.* **73,** 2469–2480.
Sanchez, A. (2007). Analysis of filovirus entry into vero e6 cells, using inhibitors of endocytosis, endosomal acidification, structural integrity, and cathepsin (B and L) activity. *J. Infect. Dis.* **196** (Suppl. 2), S251–S258.
Sanders, D. A. (2000). Sulfhydryl involvement in fusion mechanisms. *Subcell. Biochem.* **34,** 483–514.
Sandrin, V., Boson, B., Salmon, P., Gay, W., Negre, D., Le Grand, R., Trono, D., and Cosset, F. L. (2002). Lentiviral vectors pseudotyped with a modified RD114 envelope glycoprotein show increased stability in sera and augmented transduction of primary lymphocytes and CD34+ cells derived from human and nonhuman primates. *Blood* **100,** 823–832.
Saphire, A. C., Bobardt, M. D., and Gallay, P. A. (1999). Host cyclophilin A mediates HIV-1 attachment to target cells via heparans. *EMBO J.* **18,** 6771–6785.
Saphire, A. C., Bobardt, M. D., Zhang, Z., David, G., and Gallay, P. A. (2001). Syndecans serve as attachment receptors for human immunodeficiency virus type 1 on macrophages. *J. Virol.* **75,** 9187–9200.
Scarlatti, G., Tresoldi, E., Bjorndal, A., Fredriksson, R., Colognesi, C., Deng, H. K., Malnati, M. S., Plebani, A., Siccardi, A. G., Littman, D. R., Fenyo, E. M., and Lusso, P. (1997). In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nat. Med.* **3,** 1259–1265.
Schlegel, R., Tralka, T. S., Willingham, M. C., and Pastan, I. (1983). Inhibition of VSV binding and infectivity by phosphatidylserine: is phosphatidylserine a VSV-binding site? *Cell* **32,** 639–646.
Schnell, M. J., Johnson, J. E., Buonocore, L., and Rose, J. K. (1997). Construction of a novel virus that targets HIV-1-infected cells and controls HIV-1 infection. *Cell* **90,** 849–857.

Schofield, D. J., Bartosch, B., Shimizu, Y. K., Allander, T., Alter, H. J., Emerson, S. U., Cosset, F. L., and Purcell, R. H. (2005). Human monoclonal antibodies that react with the E2 glycoprotein of hepatitis C virus and possess neutralizing activity. *Hepatology* **42,** 1055–1062.

Schornberg, K., Matsuyama, S., Kabsch, K., Delos, S., Bouton, A., and White, J. (2006). Role of endosomal cathepsins in entry mediated by the Ebola virus glycoprotein. *J. Virol.* **80,** 4174–4178.

Schulz, T. F., Jameson, B. A., Lopalco, L., Siccardi, A. G., Weiss, R. A., and Moore, J. P. (1992). Conserved structural features in the interaction between retroviral surface and transmembrane glycoproteins? *AIDS Res. Hum. Retroviruses* **8,** 1571–1580.

Sessions, O. M., Barrows, N. J., Souza-Neto, J. A., Robinson, T. J., Hershey, C. L., Rodgers, M. A., Ramirez, J. L., Dimopoulos, G., Yang, P. L., Pearson, J. L., and Garcia-Blanco, M. A. (2009). Discovery of insect and human dengue virus host factors. *Nature* **458,** 1047–1050.

Shah, P. P., Myers, M. C., Beavers, M. P., Purvis, J. E., Jing, H., Grieser, H. J., Sharlow, E. R., Napper, A. D., Huryn, D. M., Cooperman, B. S., Smith, A. B., 3rd, and Diamond, S. L. (2008). Kinetic characterization and molecular docking of a novel, potent, and selective slow-binding inhibitor of human cathepsin L. *Mol. Pharmacol.* **74,** 34–41.

Shah, P. P., Wang, T., Kaletsky, R. L., Myers, M. C., Purvis, J. E., Jing, H., Huryn, D. M., Greenbaum, D. C., Smith, A. B., 3rd, Bates, P., and Diamond, S. L. (2010). A small-molecule oxocarbazate inhibitor of human cathepsin L blocks severe acute respiratory syndrome and ebola pseudotype virus infection into human embryonic kidney 293T cells. *Mol. Pharmacol.* **78,** 319–324.

Shimojima, M., Takada, A., Ebihara, H., Neumann, G., Fujioka, K., Irimura, T., Jones, S., Feldmann, H., and Kawaoka, Y. (2006). Tyro3 family-mediated cell entry of Ebola and Marburg viruses. *J. Virol.* **80,** 10109–10116.

Shimojima, M., Ikeda, Y., and Kawaoka, Y. (2007). The mechanism of Axl-mediated Ebola virus infection. *J. Infect. Dis.* **196**(Suppl. 2), S259–S263.

Shmulevitz, M., and Duncan, R. (2000). A new class of fusion-associated small transmembrane (FAST) proteins encoded by the non-enveloped fusogenic reoviruses. *EMBO J.* **19,** 902–912.

Sieczkarski, S. B., and Whittaker, G. R. (2002). Dissecting virus entry via endocytosis. *J. Gen. Virol.* **83,** 1535–1545.

Simmons, G., Gosalia, D. N., Rennekamp, A. J., Reeves, J. D., Diamond, S. L., and Bates, P. (2005). Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proc. Natl. Acad. Sci. USA* **102,** 11876–11881.

Sinn, P. L., Hickey, M. A., Staber, P. D., Dylla, D. E., Jeffers, S. A., Davidson, B. L., Sanders, D. A., and McCray, P. B., Jr. (2003). Lentivirus vectors pseudotyped with filoviral envelope glycoproteins transduce airway epithelia from the apical surface independently of folate receptor alpha. *J. Virol.* **77,** 5902–5910.

Sitbon, M., d'Auriol, L., Ellerbrok, H., Andre, C., Nishio, J., Perryman, S., Pozo, F., Hayes, S. F., Wehrly, K., Tambourin, P., *et al.* (1991). Substitution of leucine for isoleucine in a sequence highly conserved among retroviral envelope surface glycoproteins attenuates the lytic effect of the Friend murine leukemia virus. *Proc. Natl. Acad. Sci. USA* **88,** 5932–5936.

Skehel, J. J., and Wiley, D. C. (2000). Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. *Annu. Rev. Biochem.* **69,** 531–569.

Song, C., Dubay, S. R., and Hunter, E. (2003). A tyrosine motif in the cytoplasmic domain of mason-pfizer monkey virus is essential for the incorporation of glycoprotein into virions. *J. Virol.* **77,** 5192–5200.

Spies, C. P., Ritter, G. D., Jr., Mulligan, M. J., and Compans, R. W. (1994). Truncation of the cytoplasmic domain of the simian immunodeficiency virus envelope glycoprotein alters the conformation of the external domain. *J. Virol.* **68,** 585–591.

Stadler, K., Allison, S. L., Schalich, J., and Heinz, F. X. (1997). Proteolytic activation of tick-borne encephalitis virus by furin. *J. Virol.* **71,** 8475–8481.

Stein, C. S., Martins, I., and Davidson, B. L. (2005). The lymphocytic choriomeningitis virus envelope glycoprotein targets lentiviral gene transfer vector to neural progenitors in the murine brain. *Mol. Ther.* **11,** 382–389.

Stiasny, K., and Heinz, F. X. (2006). Flavivirus membrane fusion. *J. Gen. Virol.* **87,** 2755–2766.

Stieneke-Grober, A., Vey, M., Angliker, H., Shaw, E., Thomas, G., Roberts, C., Klenk, H. D., and Garten, W. (1992). Influenza virus hemagglutinin with multibasic cleavage site is activated by furin, a subtilisin-like endoprotease. *EMBO J.* **11,** 2407–2414.

Stitz, J., Buchholz, C. J., Engelstadter, M., Uckert, W., Bloemer, U., Schmitt, I., and Cichutek, K. (2000). Lentiviral vectors pseudotyped with envelope glycoproteins derived from gibbon ape leukemia virus and murine leukemia virus 10A1. *Virology* **273,** 16–20.

Supekova, L., Supek, F., Lee, J., Chen, S., Gray, N., Pezacki, J. P., Schlapbach, A., and Schultz, P. G. (2008). Identification of human kinases involved in hepatitis C virus replication by small interference RNA library screening. *J. Biol. Chem.* **283,** 29–36.

Tai, A. W., Benita, Y., Peng, L. F., Kim, S. S., Sakamoto, N., Xavier, R. J., and Chung, R. T. (2009). A functional genomic screen identifies cellular cofactors of hepatitis C virus replication. *Cell Host Microbe* **5,** 298–307.

Tailor, C. S., Nouri, A., Lee, C. G., Kozak, C., and Kabat, D. (1999a). Cloning and characterization of a cell surface receptor for xenotropic and polytropic murine leukemia viruses. *Proc. Natl. Acad. Sci. USA* **96,** 927–932.

Tailor, C. S., Nouri, A., Zhao, Y., Takeuchi, Y., and Kabat, D. (1999b). A sodium-dependent neutral-amino-acid transporter mediates infections of feline and baboon endogenous retroviruses and simian type D retroviruses. *J. Virol.* **73,** 4470–4474.

Tailor, C. S., Willett, B. J., and Kabat, D. (1999c). A putative cell surface receptor for anemia-inducing feline leukemia virus subgroup C is a member of a transporter superfamily. *J. Virol.* **73,** 6500–6505.

Tailor, C. S., Lavillette, D., Marin, M., and Kabat, D. (2003). Cell surface receptors for gammaretroviruses. *Curr. Top. Microbiol. Immunol.* **281,** 29–106.

Takeda, M., Leser, G. P., Russell, C. J., and Lamb, R. A. (2003). Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. *Proc. Natl. Acad. Sci. USA* **100,** 14610–14617.

Taplitz, R. A., and Coffin, J. M. (1997). Selection of an avian retrovirus mutant with extended receptor usage. *J. Virol.* **71,** 7814–7819.

Tatsuo, H., Okuma, K., Tanaka, K., Ono, N., Minagawa, H., Takade, A., Matsuura, Y., and Yanagi, Y. (2000). Virus entry is a major determinant of cell tropism of Edmonston and wild-type strains of measles virus as revealed by vesicular stomatitis virus pseudotypes bearing their envelope proteins. *J. Virol.* **74,** 4139–4145.

Taylor, G. M., and Sanders, D. A. (1999). The role of the membrane-spanning domain sequence in glycoprotein-mediated membrane fusion. *Mol. Biol. Cell* **10,** 2803–2815.

Temin, H. M. (1988). Mechanisms of cell killing/cytopathic effects by nonhuman retroviruses. *Rev. Infect. Dis.* **10,** 399–405.

Tremblay, C. L., Kollmann, C., Giguel, F., Chou, T. C., and Hirsch, M. S. (2000). Strong in vitro synergy between the fusion inhibitor T-20 and the CXCR4 blocker AMD-3100. *J. Acquir. Immune Defic. Syndr.* **25,** 99–102.

Tremblay, C. L., Giguel, F., Kollmann, C., Guan, Y., Chou, T. C., Baroudy, B. M., and Hirsch, M. S. (2002). Anti-human immunodeficiency virus interactions of SCH-C (SCH 351125), a CCR5 antagonist, with other antiretroviral agents in vitro. *Antimicrob. Agents Chemother.* **46,** 1336–1339.

Trotard, M., Lepere-Douard, C., Regeard, M., Piquet-Pellorce, C., Lavillette, D., Cosset, F. L., Gripon, P., and Le Seyec, J. (2009). Kinases required in hepatitis C virus entry and replication highlighted by small interference RNA screening. *FASEB J.* **23**(11), 3780–3789.

Trujillo, J. R., Rogers, R., Molina, R. M., Dangond, F., McLane, M. F., Essex, M., and Brain, J. D. (2007). Noninfectious entry of HIV-1 into peripheral and brain macrophages mediated by the mannose receptor. *Proc. Natl. Acad. Sci. USA* **104,** 5097–5102.

Turner, A., Bruun, B., Minson, T., and Browne, H. (1998). Glycoproteins gB, gD, and gHgL of herpes simplex virus type 1 are necessary and sufficient to mediate membrane fusion in a Cos cell transfection system. *J. Virol.* **72,** 873–875.

Ugolini, S., Mondor, I., and Sattentau, Q. J. (1999). HIV-1 attachment: another look. *Trends Microbiol.* **7,** 144–149.

Ujike, M., Nishikawa, H., Otaka, A., Yamamoto, N., Matsuoka, M., Kodama, E., Fujii, N., and Taguchi, F. (2008). Heptad repeat-derived peptides block protease-mediated direct entry from the cell surface of severe acute respiratory syndrome coronavirus but not entry via the endosomal pathway. *J. Virol.* **82,** 588–592.

Umashankar, M., Sanchez San Martin, C., Liao, M., Reilly, B., Guo, A., Taylor, G., and Kielian, M. (2008). Differential Cholesterol Binding by Class Ii Fusion Proteins Determines Membrane Fusion Properties. *J. Virol.*

Vaillancourt, F. H., Pilote, L., Cartier, M., Lippens, J., Liuzzi, M., Bethell, R. C., Cordingley, M. G., and Kukolj, G. (2009). Identification of a lipid kinase as a host factor involved in hepatitis C virus RNA replication. *Virology* **387,** 5–10.

Vasiljeva, O., Reinheckel, T., Peters, C., Turk, D., Turk, V., and Turk, B. (2007). Emerging roles of cysteine cathepsins in disease and their potential as drug targets. *Curr. Pharm. Des.* **13,** 387–403.

Vincent, M. J., Sanchez, A. J., Erickson, B. R., Basak, A., Chretien, M., Seidah, N. G., and Nichol, S. T. (2003). Crimean-Congo hemorrhagic fever virus glycoprotein proteolytic processing by subtilase SKI-1. *J. Virol.* **77,** 8640–8649.

Wallin, M., Ekstrom, M., and Garoff, H. (2004). Isomerization of the intersubunit disulphide-bond in Env controls retrovirus fusion. *EMBO J.* **23,** 54–65.

Wang, H., Yang, P., Liu, K., Guo, F., Zhang, Y., Zhang, G., and Jiang, C. (2008). SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res.* **18,** 290–301.

Watson, D. J., Kobinger, G. P., Passini, M. A., Wilson, J. M., and Wolfe, J. H. (2002). Targeted transduction patterns in the mouse brain by lentivirus vectors pseudotyped with VSV, Ebola, Mokola, LCMV, or MuLV envelope proteins. *Mol. Ther.* **5,** 528–537.

Weissenhorn, W., Hinz, A., and Gaudin, Y. (2007). Virus membrane fusion. *FEBS Lett.* **581,** 2150–2155.

Wengler, G. (1989). Cell-associated West Nile flavivirus is covered with E+pre-M protein heterodimers which are destroyed and reorganized by proteolytic cleavage during virus release. *J. Virol.* **63,** 2521–2526.

Wild, C., Greenwell, T., and Matthews, T. (1993). A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus-mediated cell-cell fusion. *AIDS Res. Hum. Retroviruses* **9,** 1051–1053.

Wild, C. T., Shugars, D. C., Greenwell, T. K., McDanal, C. B., and Matthews, T. J. (1994). Peptides corresponding to a predictive alpha-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection. *Proc. Natl. Acad. Sci. USA* **91,** 9770–9774.

Wilk, T., Pfeiffer, T., and Bosch, V. (1992). Retained in vitro infectivity and cytopathogenicity of HIV-1 despite truncation of the C-terminal tail of the env gene product. *Virology* **189,** 167–177.

Wong, L. F., Azzouz, M., Walmsley, L. E., Askham, Z., Wilkes, F. J., Mitrophanous, K. A., Kingsman, S. M., and Mazarakis, N. D. (2004). Transduction patterns of pseudotyped lentiviral vectors in the nervous system. *Mol. Ther.* **9,** 101–111.

Wong, A. C., Sandesara, R. G., Mulherkar, N., Whelan, S. P., and Chandran, K. (2010). A forward genetic strategy reveals destabilizing mutations in the Ebolavirus glycoprotein that alter its protease dependence during cell entry. *J. Virol.* **84,** 163–175.

Yang, Y. L., Guo, L., Xu, S., Holland, C. A., Kitamura, T., Hunter, K., and Cunningham, J. M. (1999). Receptors for polytropic and xenotropic mouse leukaemia viruses encoded by a single gene at Rmc1. *Nat. Genet.* **21,** 216–219.

Yao, Q., and Compans, R. W. (1996). Peptides corresponding to the heptad repeat sequence of human parainfluenza virus fusion protein are potent inhibitors of virus infection. *Virology* **223,** 103–112.

Yeung, M. L., Houzet, L., Yedavalli, V. S., and Jeang, K. T. (2009). A genome-wide short hairpin RNA screening of jurkat T-cells for human proteins contributing to productive HIV-1 replication. *J. Biol. Chem.* **284,** 19463–19473.

Yonezawa, A., Cavrois, M., and Greene, W. C. (2005). Studies of ebola virus glycoprotein-mediated entry and fusion by using pseudotyped human immunodeficiency virus type 1 virions: involvement of cytoskeletal proteins and enhancement by tumor necrosis factor alpha. *J. Virol.* **79,** 918–926.

York, J., Dai, D., Amberg, S. M., and Nunberg, J. H. (2008). pH-induced activation of arenavirus membrane fusion is antagonized by small-molecule inhibitors. *J. Virol.* **82,** 10932–10939.

York, J., Berry, J. D., Stroher, U., Li, Q., Feldmann, H., Lu, M., Trahey, M., and Nunberg, J. H. (2010). An antibody directed against the fusion peptide of Junin virus envelope glycoprotein GPC inhibits pH-induced membrane fusion. *J. Virol.* **84,** 6119–6129.

Young, J. K., Li, D., Abramowitz, M. C., and Morrison, T. G. (1999). Interaction of peptides with sequences from the Newcastle disease virus fusion protein heptad repeat regions. *J. Virol.* **73,** 5945–5956.

Yu, M. Y., Bartosch, B., Zhang, P., Guo, Z. P., Renzi, P. M., Shen, L. M., Granier, C., Feinstone, S. M., Cosset, F. L., and Purcell, R. H. (2004). Neutralizing antibodies to hepatitis C virus (HCV) in immune globulins derived from anti-HCV-positive plasma. *Proc. Natl. Acad. Sci. USA* **101,** 7705–7710.

Zeisel, M. B., Koutsoudakis, G., Schnober, E. K., Haberstroh, A., Blum, H. E., Cosset, F. L., Wakita, T., Jaeck, D., Doffoel, M., Royer, C., Soulier, E., Schvoerer, E., *et al.* (2007). Scavenger receptor class B type I is a key host factor for hepatitis C virus infection required for an entry step closely linked to CD81. *Hepatology* **46,** 1722–1731.

Zeisel, M. B., Barth, H., Schuster, C., and Baumert, T. F. (2009). Hepatitis C virus entry: molecular mechanisms and targets for antiviral therapy. *Front. Biosci.* **14,** 3274–3285.

Zhang, X., Fugere, M., Day, R., and Kielian, M. (2003a). Furin processing and proteolytic activation of Semliki Forest virus. *J. Virol.* **77,** 2981–2989.

Zhang, Y., Corver, J., Chipman, P. R., Zhang, W., Pletnev, S. V., Sedlak, D., Baker, T. S., Strauss, J. H., Kuhn, R. J., and Rossmann, M. G. (2003b). Structures of immature flavivirus particles. *EMBO J.* **22,** 2604–2613.

Zhao, Y., Zhu, L., Benedict, C. A., Chen, D., Anderson, W. F., and Cannon, P. M. (1998). Functional domains in the retroviral transmembrane protein. *J. Virol.* **72,** 5392–5398.

Zhou, H., Xu, M., Huang, Q., Gates, A. T., Zhang, X. D., Castle, J. C., Stec, E., Ferrer, M., Strulovici, B., Hazuda, D. J., and Espeseth, A. S. (2008). Genome-scale RNAi screen for host factors required for HIV replication. *Cell Host Microbe* **4,** 495–504.