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Picornavirus–receptor interactions

Michael G. Rossmann, Yongning He and Richard J. Kuhn

Many picornaviruses use cell-surface molecules belonging to the immunoglobulin superfamily (IgSF) as their cellular receptors. These molecules usually consist of tandem repeats of between two and five Ig-like domains whose amino-terminal domains (D1) interact with invading viruses, with their carboxy-terminal sections comprising a transmembrane and a short cytoplasmic region. Most rhino- and enteroviruses, belonging to the *Picornavirus* family, use a canyon-like feature on their surface to attach to cellular receptors. Binding into the canyon destabilizes the virus and thus initiates the uncoating process. By contrast, non-IgSF molecules, when used by picornaviruses as receptors, bind outside the canyon and do not cause viral instability.

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Most animal and bacterial viruses use specific cell-surface molecules to initiate infection. The occurrence of a particular receptor molecule on a restricted set of cell types will therefore limit cell tropism, and the variation of such a receptor among different species will limit the host range. Viruses have evolved to use a wide variety of molecules as their receptors, including proteins, carbohydrates and glycolipids [1]. Frequently, viruses can bind to, or even initiate entry into, cells by binding to a specific receptor molecule, but fail to infect the cell. Successful infection occurs only when a receptor can initiate the full viral life cycle from cell recognition to release of the genome into the cell for replication and protein synthesis. A single receptor molecule might participate in all these tasks, or one or more 'accessory' molecules could be required for different stages of viral cell entry, uncoating and synthesis of new component viral parts.

Some of the most extensive structural studies of virus–receptor interactions involve picornaviruses [2–4] (Tables 1 and 2). The external surface of rhino- and enteroviruses (two genera of *Picornaviridae*) is noteworthy for the large (~12 Å deep and ~15 Å wide) depression or 'canyon' running around each fivefold vertex. It was suggested [5] that the site of receptor attachment would involve the more conserved amino acid residues in the canyon, a site that is protected from host immune surveillance by the inability of neutralizing antibodies to penetrate far into the canyon on account of their larger cross-section. Although the basis of this 'canyon hypothesis' was challenged [6] because of the subsequent discovery that the footprints of the receptor- and antibody-binding sites overlap on the viral surface, the prediction has been substantiated by cryo-electron microscopy (cryoEM) studies (see Box 1 for a brief description of this technique) of major-group rhinoviruses bound to intercellular adhesion molecule-1 (ICAM-1 or CD54) [7–9]; coxsackievirus A21 (CAV21) bound to ICAM-1 [10]; coxsackievirus B3 (CVB3) bound to coxsackievirus–adenovirus receptor (CAR) [11]; and poliovirus (PV) bound to poliovirus receptor (PVR or CD155) [12–14]. All these receptors (Fig. 1) have similar structures in that they consist of a series of immunogloubulin superfamily (IgSF) domains, with the amino-terminal domain binding into the canyon and the carboxy-terminal sequence anchored in the host cell's plasma membrane followed by a generally small cytoplasmic domain. The structures differ in the number of domains and the degree of similarity to a constant (C) or variable (V) Ig fold.

There are, however, other receptor molecules that bind outside the canyon, namely low-density lipoprotein receptor (LDL-R) [15,16], which binds to the minor-group of rhinovirus serotypes (about ten known members), and decay-accelerating factor (DAF or CD55) ([17]; Y. He *et al.*, unpublished), which binds to some echo- and coxsackie B viruses. These receptors do not belong to the IgSF and, unlike all the IgSF-type molecules, do not initiate viral instability

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Table 1. Structures of picornaviruses complexed with their receptors[a]

Virus	Receptor	Amino-terminal domain structure	Receptor domains	Refs
IgSF receptors				
HRV14	ICAM-1	Ig intermediate domain	Five Ig domains	[8,9]
HRV16	ICAM-1	Ig intermediate domain	Five Ig domains	[7–9]
CAV21	ICAM-1	Ig intermediate domain	Five Ig domains	[10]
PV1	PVR	Ig variable domain	Three Ig domains	[12–14], b
PV2	PVR	Ig variable domain	Three Ig domains	b
PV3	PVR	Ig variable domain	Three Ig domains	b
CVB3	CAR	Ig variable domain	Two Ig domains	[11]
Other receptor types				
HRV2	LDL-R	LB domain	Eight LB domains + EGF	[16]
ECHO7	DAF	SCR domain	Four SCR domains	b
Theiler's	Sialic acid			[75]
FMDV	Heparan sulfate			[69]

[a]Abbreviations: CAR, coxsackievirus–adenovirus receptor; CAV, coxsackievirus A; CVB, coxsackievirus B; DAF, decay-accelerating factor; ECHO, echovirus; EGF, epidermal growth factor; FMDV, foot-and-mouth disease virus; HRV; human rhinovirus; ICAM, intercellular adhesion molecule; IgSF, immunoglobulin superfamily; LB, ligand binding; LDL-R, low-density lipoprotein receptor; PV, poliovirus; PVR, PV receptor; SCR, short consensus repeat; Theiler's, Theiler's murine virus.
[b]Y. He *et al*., unpublished.

and uncoating. It could be significant that there are only a few minor-group rhinoviruses, as the receptor-attachment site of these viruses is close to their fivefold vertices and thus readily accessible to neutralizing antibodies, making it more likely that these viruses would undergo a change in tissue or host specificity.

It was recognized later [18] that the canyon offers another advantage for receptor attachment, namely that the binding of the receptor triggers the uncoating process (Fig. 2). There appears to be a finely tuned interaction between an as-yet-uncharacterized 'pocket factor' (recognized as a well-defined density in crystal structures of most rhino- and enteroviruses and probably representing a cellularly derived fatty acid) that binds into a pocket below the canyon and the receptor binding into the canyon [19]. Because the binding sites of the receptor and pocket factor overlap, only one can bind at a time. The presence of the pocket factor probably stabilizes the virus, a situation which is exploited by a series of antiviral compounds [19,20] that stabilize the virus in a conformation in which it is either unable to bind to the receptor productively or to perform the essential uncoating step. Therefore, it would appear that the pocket factor stabilizes the mature virus for transport from host to host or cell to cell, but as soon as a viable receptor competes for the binding site, the pocket factor is dislodged and uncoating can proceed. This carefully balanced process can be readily disturbed either by finding a molecule that binds better than the natural pocket factor and therefore cannot be dislodged, or by altering the receptor-binding surface to decrease the affinity of the receptor for the virus to the extent where the receptor is unable to dislodge the pocket factor [18,21].

Picornaviruses and IgSF receptor molecules

Picornaviruses are small, icosahedral, non-enveloped viruses with a plus-sense RNA genome. They are among the most common animal virus pathogens and include human rhinoviruses (HRV), PV, coxsackieviruses, foot-and-mouth disease viruses (FMDV) and hepatitis A virus (HAV). High-resolution

Table 2. Known picornavirus receptors[a]

Virus	Receptor	Refs
Major-group HRV (90 serotypes)	ICAM-1	[76,77]
Minor-group HRV (10 serotypes)	LDL-R	[15]
PV (3 serotypes)	PVR	[53]
CVB (6 serotypes)	CAR, DAF	[17,43]
CAV21	ICAM-1	[78]
CAV9	$\alpha_v\beta_3$ integrin	[66]
ECHO (10+ serotypes)	DAF	[17]
ECHO (serotypes 1 and 8)	VLA-2 ($\alpha_2\beta_1$ integrin)	[79]
HAV	HAVcr-1	[80]
FMDV (7 serotypes)	$\alpha_v\beta_3$ and $\alpha_v\beta_6$ integrin, heparan sulfate	[66–68]
Theiler's	Sialic acid	[75]

[a]Abbreviations: CAR, coxsackievirus–adenovirus receptor; CAV, coxsackievirus A; CVB, coxsackievirus B; DAF, decay-accelerating factor; ECHO, echovirus; FMDV, foot-and-mouth disease virus; HAV, hepatitis A virus; HAVcr, HAV cellular receptor; HRV; human rhinovirus; ICAM, intercellular adhesion molecule; LDL-R, low-density lipoprotein receptor; PV, poliovirus; PVR, PV receptor; Theiler's, Theiler's murine virus; VLA, very late antigen.

Box 1. Cryo-electron microscopy

Many of the results discussed in this review are dependent on the fairly new technology called 'cryo-electron microscopy' (cryoEM). In this technique, aqueous samples are rapidly frozen to form vitrous ice. This process introduces a minimum of artifacts in the specimen and allows for longer electron exposure with minimal radiation damage. Each image formed by the electron microscope is a projection of the sample along the direction of the electron beam. By combining the 2-D projections of hundreds or even thousands of such randomly oriented particles, it is possible to reconstruct the 3-D image of the particle. The greater the number of projections, the better will be the resolution of the final reconstruction [a] within the limits of the available map.

Reference

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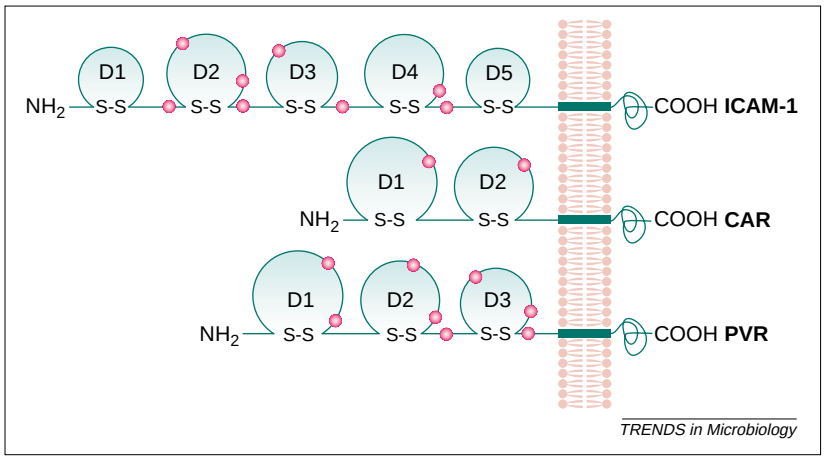

Fig. 1. Diagrammatic structures of the immunoglobulin superfamily (IgSF) molecules intercellular adhesion molecule-1 (ICAM-1); coxsackievirus–adenovirus receptor (CAR); and poliovirus receptor (PVR) used as receptors by human rhinoviruses (HRVs) and coxsackievirus A21 (CAV21); coxsackie B viruses (CVB); and polioviruses (PV), respectively.

structures have been determined of a few serotypes of many of these different viruses, starting with the determination of HRV14 [5] and PV1 [22]. Virions are ~8.5 × 10^6 Da in mass, have an external diameter of ~300 Å, and contain 60 protomers, each of which is made up of four polypeptides, VP1–VP4. Sixty copies of each of the first three of these viral proteins reside on the exterior of the virus and make up its icosahedral protein shell.

The various IgSF molecules that can act as a receptor for many of the rhino- and entero-picornaviruses (Table 2) are type I transmembrane glycoproteins whose extracellular regions consist of between two to five Ig domains. In all these receptors, the amino-terminal domain, D1, contains the virus-recognition site. Hence, virus attachment occurs at a site on the receptor that is distal from the plasma membrane. This property could be important for successful initiation of infection of cells by viruses and could reflect the ability of the amino-terminal Ig domain to penetrate into the picornavirus canyon. The nature of these long molecules and the importance of the amino-terminal domain to their cellular function is also reflected in their cell-adhesion properties [23]. In this review, we confine ourselves primarily to the interaction of IgSF molecules with picornaviruses.

IgSF domains have a structure that consists of a β-barrel fold in which all the β-strands (A to G) run parallel or antiparallel to the long axis of the domain. The detailed structure of the Ig fold can be either that of a V domain or a C domain, or in an intermediate (I) conformation [24].

Cell entry and uncoating are initiated when an appropriate IgSF receptor molecule binds to the relevant virus. In many cases, purified soluble receptor molecules, as well as the membrane-anchored receptors, convert infectious virions to altered ('A') particles (135S) [25–27]. VP4 is absent in A particles, and the amino terminus of VP1 is externalized [28]. *In vitro*, slightly longer incubation leads to the formation of 80S particles, which are devoid of the genomic RNA. It is uncertain, however, whether the 135S and 80S particles are real intermediates in the *in vivo* uncoating pathway [29–31].

HRV14, HRV16 and CAV21 interaction with ICAM-1

The interactions of various ICAM-1 fragments, all containing the two amino-terminal domains, have been investigated when complexed with the major-group HRV serotypes 14 and 16 and with CAV21 (Fig. 3). The fragments differ in their lengths, containing between two and five extracellular domains, and their degree of glycosylation. Three of the four glycosylation sites of the D1D2 fragment can be eliminated while still retaining the ability to bind to virions and to block cell infection [9]. The structures of unglycosylated ICAM-1 fragments have been determined by X-ray crystallography [9,32], whereas the structures of transient, unstable complexes have been determined by cryoEM [7,8,10]. Difference maps between complexes using the glycosylated and unglycosylated ICAM-1 fragments were used to determine the positions of the carbohydrate sites. The position of these sites permitted docking of the known atomic structures of the HRVs and CAV21 with the ICAM-1 receptor, within the ~21 Å resolution limits imposed by the lower-resolution cryoEM results.

ICAM-1 was found to bind centrally in the canyon in HRV14, HRV16 and CAV21, as had been predicted

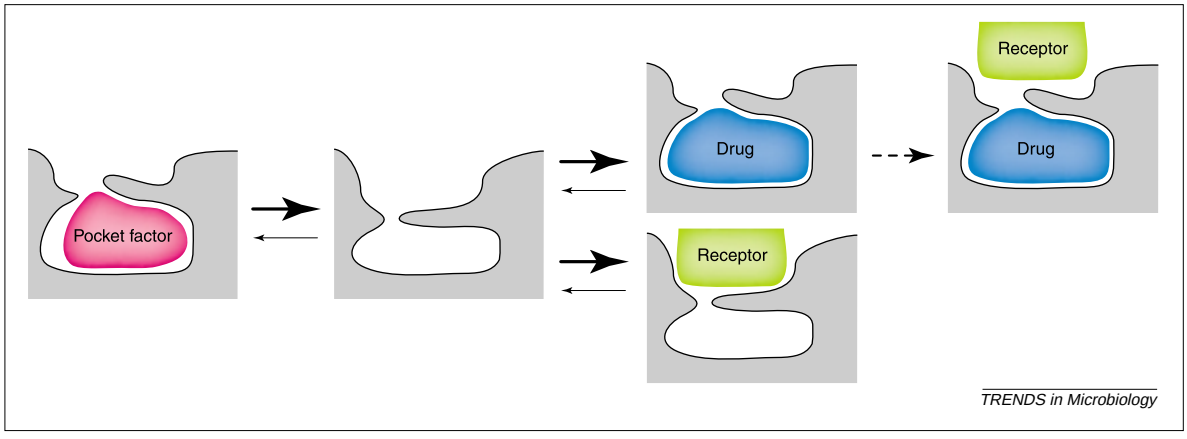

Fig. 2. Diagrammatic representation of the proposed competition between binding of pocket factor or drug (magenta or blue, respectively) into the VP1 binding pocket and receptor (green) into the canyon. (Adapted, with permission, from [18].)

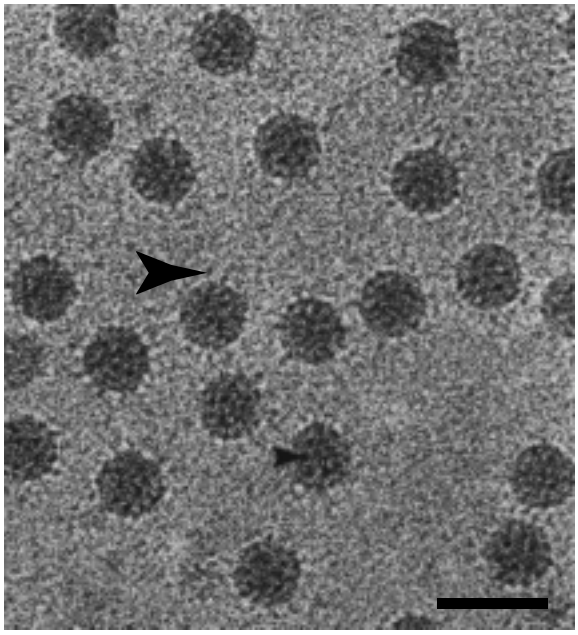

Fig. 3. Cryo-electron microscopy (cryoEM) of human rhinovirus 16 (HRV16) particles complexed with intercellular adhesion molecule-1 (ICAM-1) D1D2. The images were recorded at a magnification of ×47 500 and with an electron dose of ~20 electrons per Å^2. The D1D2 molecules (the two amino-terminal domains of ICAM-1) are seen edge-on at the periphery of the virions (large arrowhead) or end-on in projection (small arrowhead). (Reprinted with permission from [7].)

by the canyon hypothesis. However, it did spread out over the canyon rims into regions associated with the binding of neutralizing antibodies [6,33]. The overlap of receptor- and antibody-binding sites might suggest a conflict with the canyon hypothesis, yet the residues identified as being important in antibody binding to HRV14 [34,35] are well outside the canyon and therefore might not be important for receptor binding. The converse situation, demonstrating that residues in the center of the canyon are the most important for receptor binding, was accomplished by Colonno [36] and by Bergelson for CAR (J.M. Bergelson, unpublished; see below).

The orientation of the ICAM-1 molecule with respect to the virus surface is almost exactly the same for ICAM-1 binding to HRV14 and HRV16, as well as a mutant 'Kilifi' ICAM-1 [37], which binds only to HRV14 and not to HRV16. By contrast, the orientation of ICAM-1 binding to CAV21 is quite different [10], as is the orientation of each known IgSF receptor with respect to its particular virus (Fig. 4). This is rather surprising as the difference in amino acid sequence between HRV16 and HRV14 is as great as that between HRV16 and any enterovirus [38].

The tip of ICAM-1 D1 and the canyon wall and floor of HRV16 and HRV14 show extensive shape and charge complementarity [8]. HRVs bind to ICAM-1 but not to other homologous molecules such as ICAM-2 or ICAM-3. This specificity has been rationalized by the crystallographic and sequence analysis of the BC, DE and FG binding loops in domain D1, which differ in sequence among the different ICAM-1s [9].

Various lines of investigation [39,40], as well as the instability of the HRV–ICAM-1 virus–receptor complexes, suggest that the structures observed in the picornavirus–IgSF receptor complexes represent an initial recognition event. Only subsequently is the receptor likely to bind deeper within the canyon and thereby possibly compete-out the pocket factor in the VP1 pocket [19]. Loss of pocket factor presumably leads to virus destabilization and progressive disassembly with release of the genomic RNA. It has been speculated [8] that the natural breathing of picornaviruses [41] might facilitate receptor binding to both the north and south walls of the canyon, thus opening a channel to permit the externalization of VP4, the amino end of VP1 and, eventually, the RNA.

Interaction of CAR with CVB3

The six coxsackievirus B serotypes and many adenoviruses share CAR as a common receptor on human cells [42–45]. Because these two virus families are unrelated, their receptor specificity must have evolved independently. CAR is a 45 kDa membrane glycoprotein with two Ig-like extracellular domains (D1 and D2), a transmembrane domain and a 107 amino acid cytoplasmic domain [43–45]. CAR is expressed in many tissues and is highly conserved between mice and humans. It might have a function in cell adhesion [46], but its precise physiological role remains unclear. The role of CAR in CVB and adenovirus infection is different. For CVBs, CAR can function in both attachment and infection [45]. For adenoviruses, however, the major function of CAR is to mediate the initial attachment of the virus to the cell surface, whereas subsequent entry of virus into cells is mediated by an integrin [47,48].

CryoEM reconstructions were made of images of CVB3 complexed with either full-length human CAR, with the ectodomain (D1D2) of human CAR, or with the D1 domain alone. The full-length CAR showed domain D1 bound into the CVB3 canyon and the transmembrane region of CAR buried in what was assumed to be a lipid-like structure produced by the NP40 detergent used to solubilize CAR. As the structures of CVB3 [49] and CAR D1 [50,51] were both known, it was possible to obtain a fairly accurate fit of these atomic structures to the lower-resolution cryoEM density map of the CVB3–CAR complex. Recently, it has been observed that zebrafish CAR, as well as human and mouse CAR, all bind to CVB3 (J.M. Bergelson, pers. commun.). Residues in the CAR footprint on the CVB3 surface that are completely conserved among these three animals are all clustered into the canyon region. Those residues in the CAR footprint that are not conserved are almost exclusively confined to the south rim of the canyon and belong to the hypervariable 'puff' region of VP2 between βE and αB (Fig. 5). This region constitutes the neutralizing immunogenic II (NImII) site for the binding of neutralizing antibodies in HRV14 [5] and PV [52].

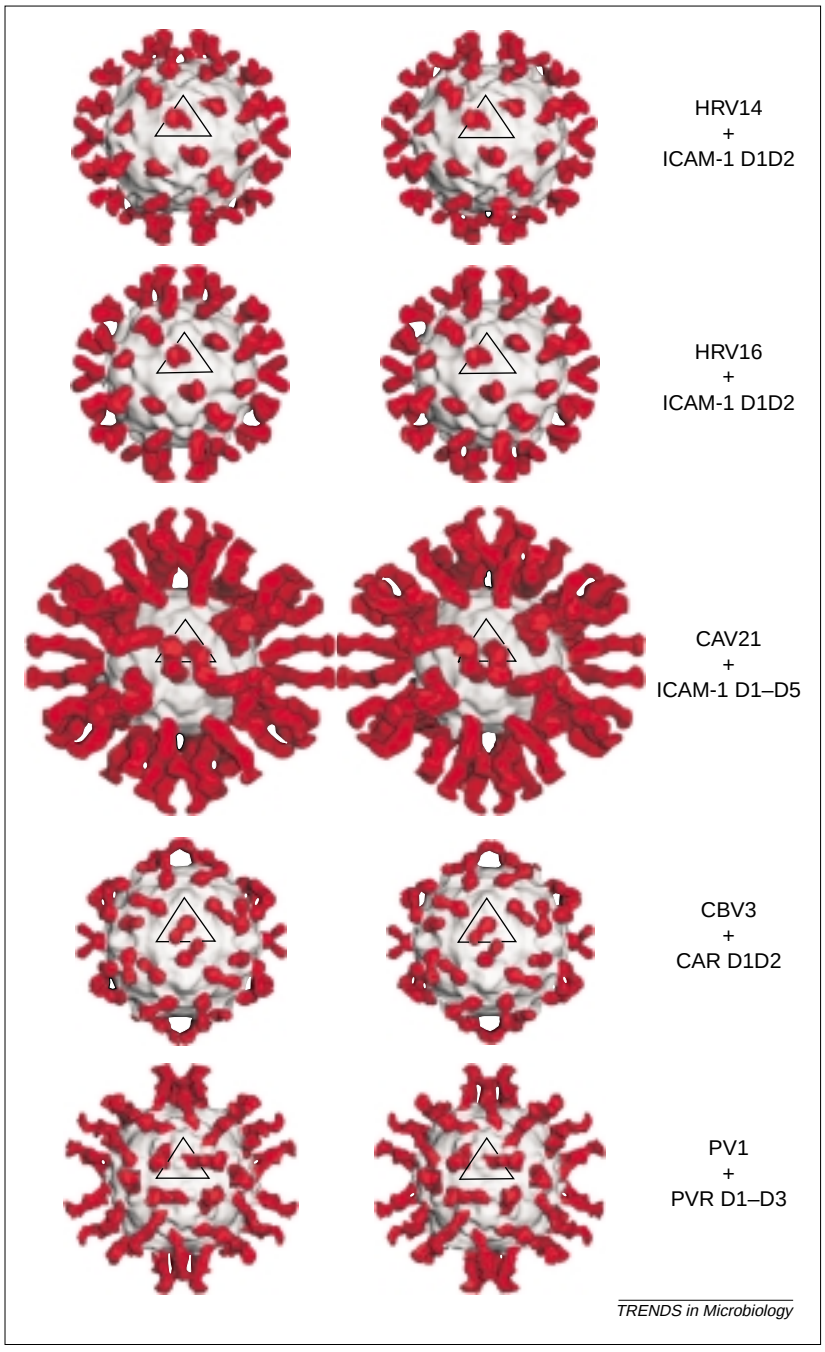

Fig. 4. Stereo diagrams of cryo-electron microscopy (cryoEM) reconstructions showing complexes of virus with soluble receptor molecules (red). The triangle demonstrates the limits of one icosahedral asymmetric unit. See the inset in Fig. 5 to locate the canyon and icosahedral symmetry elements. (Adapted, with permission, from [8]; © 1999, Oxford University Press, [10,11]; © 2001, Nature America Inc. http://www.nature.com, and [12].)

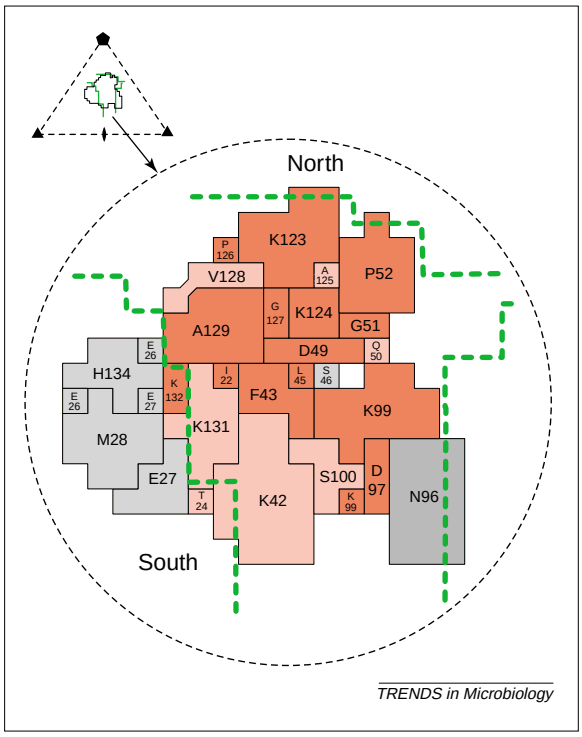

Fig. 5. The footprint that coxsackievirus B3 (CVB3) makes on coxsackievirus–adenovirus receptor (CAR) in the formation of the virus–receptor complex [11]. CAR residues that bind within the canyon are between the two dashed green lines that limit the rims of the viral canyon. The CAR residues are labeled with their amino acid identity and sequence number. Residues in gray are variable between human, mouse, and zebrafish CAR. Residues in red are completely conserved. Residues in pink sustain only conservative changes. Note that the residues binding into the canyon are far more conserved than those binding to the south or north canyon rims. Inset is shown an icosahedral asymmetric unit of the virus to identify the position of the CAR footprint.

Thus, the important residues for binding the receptor are in the canyon, whereas residues outside the canyon are hypervariable and available for attack by antibodies, consistent with the canyon hypothesis.

CAR uses the distal end and the A-G side of D1 for binding to CVB3, whereas it uses the C, C′ and C″ strands and the FG loop on the other side of D1 for binding to adenoviruses [48,50]. The overlapping binding region of CVB3 and adenovirus on CAR is located at the beginning of strand C and the FG loop, accounting for the competitive binding of these viruses to HeLa cells [42] (Fig. 6).

PV Interaction with PVR

All three PV serotypes recognize the same cellular receptor molecule, PVR [27,53,54]. PVR is a transmembrane glycoprotein with three Ig domains forming the extracellular component. As in all other IgSF molecules used as viral receptors, the amino-terminal domain D1 provides the virus-attachment surface. Comparison of glycosylated and deglycosylated forms of PVR have helped to orientate a model of PVR in cryoEM difference maps. As is the case for the binding of ICAM-1 to different rhinovirus serotypes, the mode of binding of PVR to each of the three PV serotypes is very similar ([12]; Y. He *et al.*, unpublished). However, to date, the structure of PVR has not been determined. Thus, fitting the PVR structure to cryoEM maps has to be based upon homology-built models, resulting in differences of interpretation between Belnap *et al.* [13] on the one hand and He *et al.* [12] or Xing *et al.* [14] on the other.

Although domain D1 of PVR binds into the canyon at much the same site [12] as other IgSF receptor molecules bind to other picornaviruses, its orientation with respect to the virus surface is rather

different (Fig. 4). Indeed, domain D1 does not have its long axis radial to the viral surface, but can be roughly described as being tangential (Fig. 7).

The north side of the PV canyon forms a hydrophobic surface that interacts with an equally extensive hydrophobic surface on PVR. The two PV VP1 loops that form the north rim of the canyon had been implicated correctly by mutagenesis studies as being involved in binding PVR [55]. The south rim of the canyon forms a hydrophobic surface consisting, in part, of residues in the VP2 'puff' region. The corresponding binding regions of PVR were also identified in mutagenesis studies [56]. Similarly, residues involved on the floor of the canyon had been recognized by mutagenesis before the cryoEM results [56,57]. The only charge interactions between PV and PVR occur in the additional surface created by the tangential positioning of domain D1, causing it to associate with the southeast rim of the canyon.

Interaction of non-IgSF cell-surface molecules

Enteroviruses and rhinoviruses, although classified as belonging to different genera, all have a canyon and tend to use IgSF cell-surface molecules as their receptors. A likely addition to this group is HAV whose structure is not known, but which uses the IgSF HAV cellular receptor-1 (HAVcr-1) as a receptor [58]. However, in some instances, these viruses can use non-IgSF molecules that do not bind into the canyon as receptors. The two documented cases are very-low-density lipoprotein receptor (VLDL-R), which is used by the minor-group of HRV serotypes [15,16], and DAF (or CD55), which is used by some echoviruses ([17]; He *et al.*, unpublished) and some coxsackie B viruses [59] (both these viruses belong to the enterovirus genus of picornaviruses). VLDL-R binds north of the canyon close to the icosahedral fivefold vertices (Fig. 8a), whereas DAF binds south of the canyon around the icosahedral twofold axes (Fig. 8b). Unlike all the IgSF receptors, these receptors do not cause viral instability upon binding [26,60] (IgSF receptors when complexed with their respective viruses can cause the virus to fall apart within minutes or hours at room temperature in the preparation of cryoEM samples) and thus do not, in themselves, trigger uncoating. However, their recruitment can trigger the aggregation of other receptor molecules [59,61] or they could trigger endocytosis followed by a lowering of pH in endosomal vesicles.

Cardioviruses have an apparent surface depression corresponding to the central portion of the canyon, identified as a 'pit' [62]. It is only 'apparent' because the GH loop of VP1 is disordered, but when ordered at low pH [63], the pit is filled. There is evidence that the cellular receptor binds into the pit [63]. The receptor might be sialic acid [64] or vascular cell adhesion molecule-1 (VCAM-1) [65]. Although the pit is in essentially the same site as part of the canyon, there is no evidence for the presence of a pocket factor underneath the pit, nor

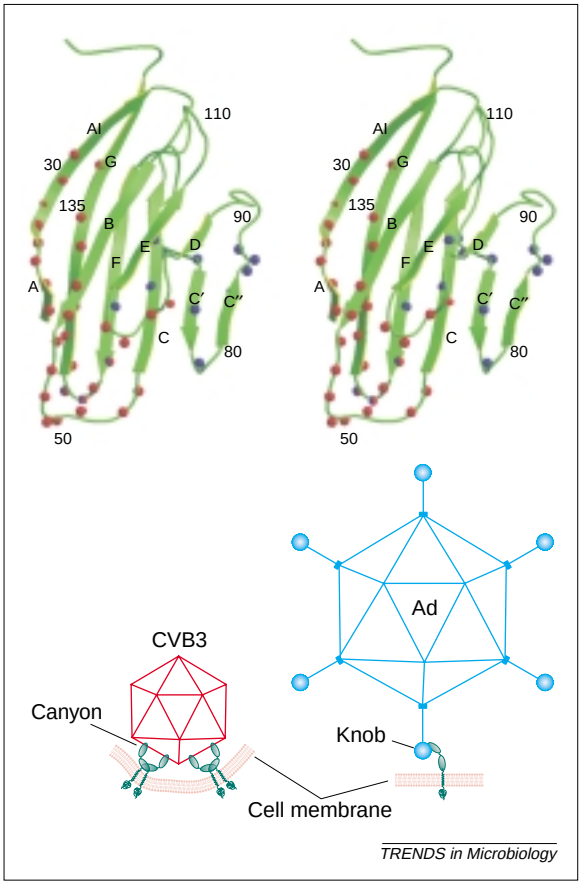

Fig. 6. Top: Stereo diagram showing a ribbon diagram of coxsackievirus–adenovirus receptor (CAR) D1. The amino acids in the coxsackievirus B3 (CVB3)–CAR interface are indicated with red spheres and the CVB3–adenovirus interface with blue spheres. Bottom: Schematic diagram of the modes by which CAR (green) binds to CVB3 (red) and adenovirus (blue). The suggested membrane curvature is speculative. (Reprinted with permission from [11]; © 2001, Nature America Inc. http://www.nature.com)

that binding of sialic acid fragments to cells causes viral instability.

Aphthoviruses (FMDVs) possess an RGD motif in the disordered GH loop of VP1, suggesting that this motif might be used for attachment to integrins. Indeed, most FMDV strains can use the vitronectin receptor, an ($\alpha_V\beta_3$) integrin [66] or an ($\alpha_V\beta_6$) integrin [67]. Other serotypes of FMDV can use heparan sulfate [68] or oligosaccharides [69] as a receptor. However, FMDV does not have a canyon [70], nor does receptor attachment seem to cause viral instability.

Outlook for the future

With the advent of cryoEM reconstructions that now can achieve ~12 Å resolution almost routinely and 6 Å resolution occasionally, it becomes possible to examine many of the cryoEM virus–receptor complex reconstructions at much higher resolution (Fig. 3). This will make it possible to define the amino acid residues at the virus–receptor interface more accurately to ascertain whether the elusive pocket factor is still resident in its binding pocket. Such results will also stimulate extensive

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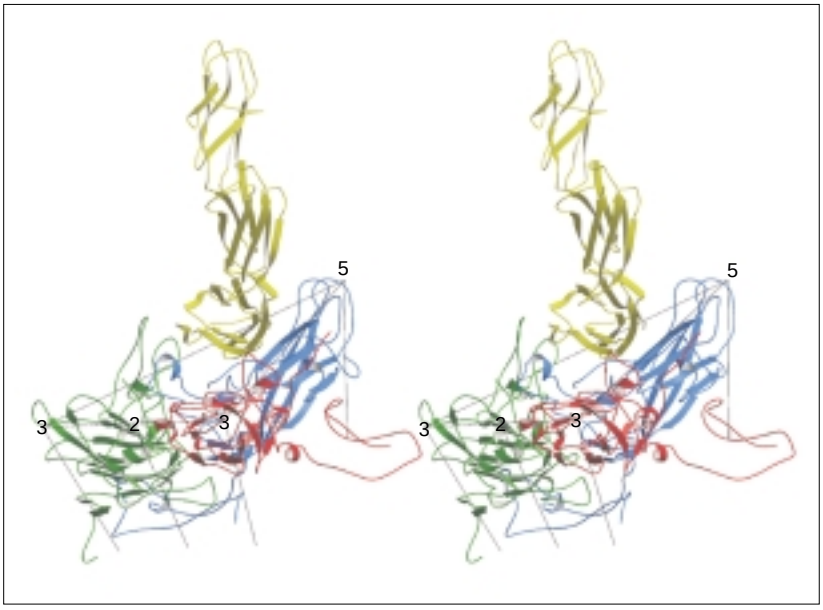

Fig. 7. Stereo view of poliovirus receptor (PVR) (yellow) docked onto poliovirus (PV). VP1, VP2 and VP3 are colored blue, green and red, respectively. (Reprinted with permission from [12].)

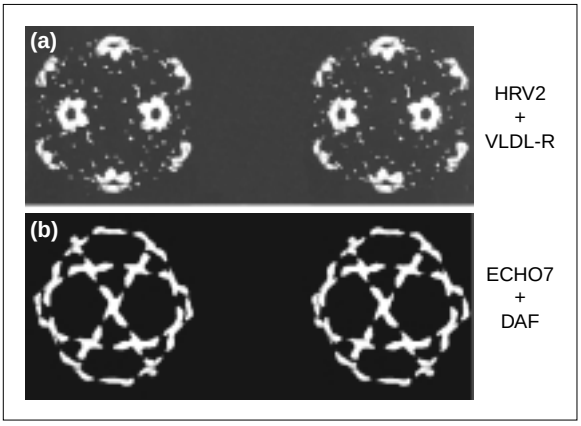

Fig. 8. Stereo diagrams showing difference density corresponding to receptor binding of very-low-density lipoprotein receptor (VLDL-R) to human rhinovirus 2 (HRV2) (a) and of decay-accelerating factor (DAF) to echovirus serotype 7 (ECHO7) (b). (Adapted with permission from [16]; © 2000, Oxford University Press.)

mutagenesis of the interface residues both in the virus and the receptor. Thus, the proposed mechanism by which canyon binding receptors initiate uncoating should become more firmly established.

Less is known or understood about the non-canyon-binding receptors such as DAF, VLDL-R, very late antigen-2 (VLA-2) or other integrins. Where do the integrins bind to entero- or aphthoviruses? Is a secondary accessory receptor required for cell entry? Is it merely the pH change in endosomal vesicles that is sufficient for uncoating? Why do the canyon binding receptors need a special trigger if all that is necessary is a pH change?

The interaction of HIV with CD4 and the chemokine receptors has had considerable attention and seems to have some properties similar to the canyon binding interactions for picornaviruses [71]. Of special interest for HIV and other enveloped viruses is the triggering of fusion with the host's cell membrane subsequent to cell attachment. A significant amount of information is available for HIV, myxo-, and orthomyxoviruses, in part through the efforts of Don Wiley, John Skehel [72], Peter Kim [73], and their colleagues. In addition, the simpler enveloped viruses, such as toga- and flaviviruses, are just starting to make a contribution towards the understanding of early events in viral infection.

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