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Review

Viral channel proteins in intracellular protein–protein communication: Vpu of HIV-1, E5 of HPV16 and p7 of HCV☆

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ABSTRACT

Viral channel forming proteins are known for their capability to make the lipid membrane of the host cell and its subcellular compartments permeable to ions and small compounds. There is increasing evidence that some of the representatives of this class of proteins are also strongly interacting with host proteins and the effectiveness of this interaction seems to be high. Interaction of viral channel proteins with host factors has been proposed by bio-informatics approaches and has also been identified experimentally. An overview of the interactions with host proteins is given for Vpu from HIV-1, E5 from HPV-16 and p7 from HCV. This article is part of a Special Issue entitled: Viral Membrane Proteins — Channels for Cellular Networking.

Contents

1. Introduction

Viruses hijack the cell just using their proteins and DNA/RNA. By steering the cell viruses improve their own replication. The improvement could be an alteration of ion and substrate concentrations in cellular sub-compartments, the generation of additional cellular sub-

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compartments, the suppression of cellular defense system and the stimulation of cell growth factors. The virus does not produce viral hormones to conduct these tasks. The only known mechanism of retroviral proteins is through viral-host protein–protein interactions.

Viral globular and membrane proteins are used for reproduction and steering of host factors. For the latter, interaction is not only between extramembrane parts of viral and host proteins but also between the transmembrane domains. The membrane allows electrochemical gradients to be generated. With membrane proteins the stored energy can be harvested to drive viral replication. A large number of viruses are identified to harbor short membrane proteins with one to three transmembrane spanning domains able to exploit electrochemical or substrate gradients in the host cell. A major characteristic of these proteins is that they form oligomers similar to their cellular ion channel companions but are very much smaller in size. It is these two issues, cellular steering of host proteins and channel formation, within the lipid membrane which make these proteins a highly attractive topic to investigate. In addition some of these proteins have emerged already as highly important drug targets.

Investigations of viral membrane proteins have seen the emergence of research focusing on 'viral channel forming proteins' [1,2], also named as 'viroporins' [3,4], which are identified to render lipid membranes permeable to ions and substrates [5]. One of these proteins, M2 from influenza A, has been a major antiviral drug target. Stimulated by the importance of this protein, a successful search for viral channel proteins has been intensified and lead to an increasing number of potential candidates [2,6]. Viral channels are formed by either bitopic, or polytopic membrane proteins with 2 and 3 TMDs (Fig. 1). For most of these viral channel proteins their role within the infectivity cycle is yet to be identified.

Within the family of viral channel proteins, Vpu from HIV-1 is one of the most controversial channel proteins. Despite the identification of this protein to form channels in artificial bilayers [7] and to increase conductivity in infected *Xenopus* oocytes [7,8], its role as a channel has not yet been identified (see K. Strebel, this issue). At the same time of its identification to form ion channels, its role to down-regulate receptor proteins has been found [9,10] (Fig. 1). Within recent years the number of host factors been manipulated by Vpu has risen. With the classification of E5 from HPV to be a viral channel protein in 2012 [11], there seems to be a most recent 'novel' member of the channel family. Before that time in 2012, E5 has actually been known for a long time for its role as a host factor manipulator interacting with host proteins.

Thus the question arises, are the virus-encoded channel proteins a single task-unit or can they adopt multiple roles? It is still not clear whether structurally distinct features such as orientation or state of oligomerization of the proteins are necessary to fulfill the tasks or whether there is equilibrium between the different features (Fig. 2).

In this review the focus is on Vpu of HIV-1, E5 of HPV and p7 of HCV for which the interactions with host factors have been experimentally identified or have been proposed by bioinformatics approaches.

2. Vpu of human immunodeficiency virus type 1

Vpu is a bitopic 81 amino acid type I membrane protein expressed in the late life cycle of human immunodeficiency virus type 1 [12,13]. Its role in the infectivity cycle is to enhance particle release *via* interaction with host factors and also *via* channel functionality (see recent reviews [2,4]). Its short N terminal extramembrane part of a few amino acids leads into a transmembrane domain (TMD) which is highly hydrophobic, consisting of a single leucine and multiple isoleucines and valines. It harbors a single hydrophilic residue, Ser-24, proceeded by Trp-23. The sequence then enters into the cytoplasmic domain *via* an EYR-motif which probably functions as a hinge between the two domains. Serines at positions 52 and 56 are identified to be phosphorylated [10].

The capability to form channels has been discovered using electrophysiological measurements on artificial lipid bilayers [7]. Independently channel activity has been confirmed also by other groups using the same techniques [14]. The protein has been identified to generate a weakly selective pore [7,14–16]. Mutations within the TMD of the protein are altering channel kinetics [7,16]. In particular, mutating Ser-24 leads to an abrogation of channel activity of a respective peptide corresponding to the TMD of Vpu [16]. Evidence of Vpu mediated leakage of small molecules from *Escherichia coli* has also been reported [17]. Physiological measurements on Vpu expressed in *Xenopus* oocytes and 293T cells support the ability of the protein to form channels [7,18].

Summarizing the evidences, up to now all channel characteristics are based on peptides and full length proteins reconstituted into artificial lipid membranes or when over expressing a Vpu containing plasmid in cells.

Identification of channel formation of Vpu has had implications on the computational modeling of the protein. The first computational model of the membrane embedded part of Vpu is a pentameric assembly of the helical TMDs [19] (Fig. 1A). The helical motif has been chosen in analogy to the M2 channel from influenza A. With the bundle model ion selectivity has been proposed on a structural level by calculating potential energy profiles of Na and Cl ions along the bundle axis. These data have later been confirmed when calculating the potential of mean force for monovalent ions being pulled through a pentameric TMD bundle [20]. In another study combining bilayer recordings with computational modeling a tetrameric bundle, based on modeling the TMD, has been discarded [21]. Molecular dynamics simulations have also revealed that hexameric bundles of the TMDs do not remain in a pore like structure when simulated for several nanoseconds [22]. Also other assemblies, pentameric and tetrameric bundles, do not remain in a bundle structure when simulated for longer time scales [23].

2.1. Interaction with CD4

Almost parallel to the emerging evidences that Vpu forms a channel it has been discovered that Vpu down-regulates the receptor protein CD4 (Fig. 1A) from the infected cell [9,24–26] reviewed in [25] (Table 1). For the down-regulation, the two phosphorylation sites, Ser-52 and Ser-56, have been found to be essential as well as an interaction with the cytoplasmic domain of CD4 [27]. Without the phosphorous groups, Vpu is still able to contact CD4, but down-regulation is hampered [10,24,28]. Mutation studies on both CD4 and Vpu have identified structural and sequence specific motifs on both proteins which seem to be essential for interaction. It has been reported that a stretch in CD4, Arg-396 to Lys-417, is predicted to be helical and essential for phosphorylation-dependent down-regulation of CD4 [29]. Solution NMR spectroscopic data of a CD4 mutant construct recorded in micelles confirm a helical motif of the membrane-proximal cytoplasmic stretch Ala-404 to Leu-413 of CD4 [30]. A deletion mutant of CD4 in this region identifies the stretch, KRLLSEKKT, in this cytoplasmic domain to be essential for the Vpu binding [31]. Substitution mutations in this region have not resulted in abrogation of Vpu binding. Therefore, it has been suggested that the mutations may have not led to a derangement of the helical motif and that this motif is important for proper interaction with Vpu. As far as Vpu is concerned it is reported that both cytoplasmic helices are involved in CD4 down-regulation [31]. Especially when short specific amino acid sequences within the first cytoplasmic helix of Vpu are mutated, e.g. the short stretches YRK or DRLI, as well as the single mutation L63P, Vpu loses its capability to down-regulate CD4. Since all the mutations are generated to disrupt the helical motif it is concluded that for Vpu-mediated CD4 down-regulation a helix–helix interaction motif is essential.

The structure of the cytoplasmic domain of Vpu as a whole and also individual parts of this domain have been deciphered by various authors using different techniques such as solution [32–35] and solid state NMR spectroscopy [36,37], as well as CD spectroscopy [32,35]. The current structural model of Vpu is comprised of a helical TMD, linked to the

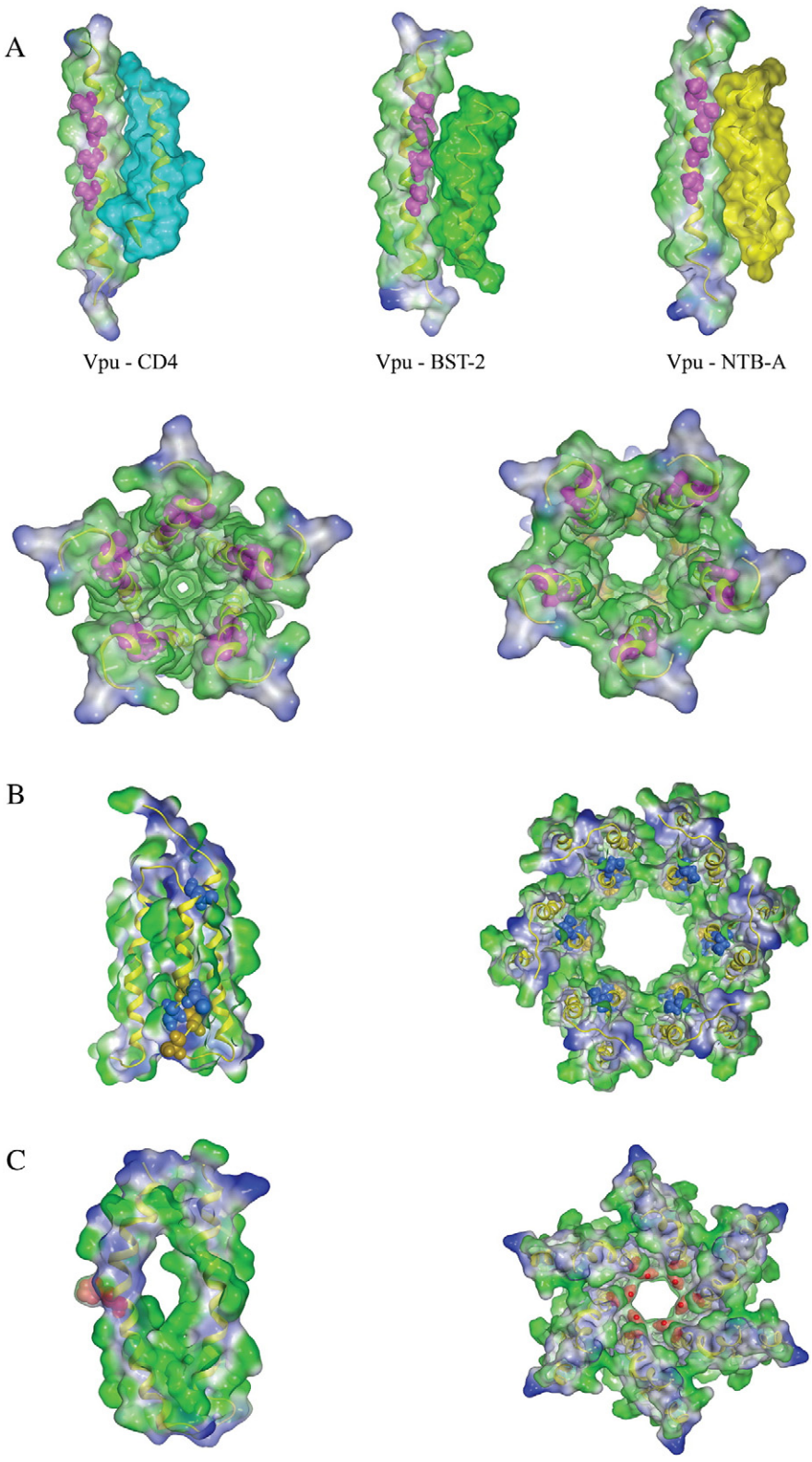

Fig. 1. (A) Computational models of the first 32 amino acids including the transmembrane domain (TMD) of the bitopic Vpu (Vpu_{1-32}) from HIV-1 (Vpu HV1H2) [23]. The TMD of Vpu_{1-32} is modeled in its interacting form with the TMD of the receptor protein CD4 (blue, left), BST-2 (green, middle) and NTB-A (yellow, right) in the upper panel. The lower panel shows potential pentameric assemblies of Vpu_{1-32} as a hydrophobic pore (left) and a pore with hydrophilic residue serine (yellow) facing the inside of the bundle (right). The putative monomeric form of E5 protein seen from the side of TMD2 (B). Serine residues (yellow) and threonines (blue) are shown in van der Waals spheres. A putative hexameric bundle with TMD2s facing the pore is also shown (right). (C) The polytopic channel protein p7 from HCV is shown as a monomer with its two TMDs aligned antiparallel (left) and in a putative hexameric bundle (right). The models highlight the histidine residues (red), which are thought to face the lumen of the pore. All models are generated with surface mode of MOE software suite coloring hydrophilic residues in green and hydrophilic residues in blue. The backbones of the TMDs are shown in light yellow.

Table 1
Host proteins interacting with the viral proteins Vpu of HIV-1, E5 of HPV-16 and p7 of HCV. Host proteins are identified by experiments or bioinformatics tools [96]. HPRD ID: human protein reference database identification number; SVM: support vector machine; ELM: eukaryotic linear motif (http://elm.eu.org/).

Host protein (HPRD IDs, ELM)	Number of TMDs of host protein	Binding region of viral protein	Experimental and computational methods used	Reference
Vpu				
CD4	1	Cytoplasmic region	Immunoprecipitation	[121,27]
TASK	4	TMD	Sequence similarity, coimmunoprecipitation	[38]
BST-2	1	TMD	NMR, co-immunoprecipitation, western blot analysis, flow cytometry	[41,42,44,45,50]
NTB-A	1	TMD	Immunoprecipitation, western blot analysis	[53]
MHC II/CD74	1	Cytoplasmic region	Yeast two hybrid assay, coimmunoprecipitation	[55]
From bioinformatics				
ELM: TRG_LysEnd_APsAcLL_1	Found in type I transmembrane proteins		Comparison of ELMs	[58]
E5				
Karyopherin β3	–	10 amino acids at the C-terminus	Immunoprecipitation and western blot analysis	[92]
COX-2, XBP-1 and IRE1a	IRE1a: 1 TMD	Not defined	RT-PCR and cDNA microarray	[93]
16-kDa protein of vacuolar H^+-ATPase	1, (vacuolar H^+-ATPase: 7 TMDs)	Residues 41 to 54	PCR and co-immuniprecipitation	[81]
EVER1, EVER2, ZnT-1	EVER1: 10 TMDs EVER2: 8 TMDs ZnT-1: 6 TMDs	Not defined	Yeast two hybrid assay	[122]
Calnexin	1 TMD	1st TMD	Co-immunoprecipitation	[86]
ErbB4	1 TMD	Not defined	Co-immunoprecipitation western blot analysis, RT-PCR, mammalian two hybrid experiment	[78]
Calpactin I	–	20 aa at C-terminus, TMD3		[89]
Bap31	3 TMDs	Last 10 aa of 3rd TMD	Yeast two hybrid assay	[91]
MHC I, HLA-I (00826)	1 TMD	With leucine pairs in 1st TMD	RT-PCR, western blot analysis, co-immunoprecipitation, flow cytometry	[85,96]
EGFR		Part of TMD3	Western blot analysis	[82]
From bioinformatics				
MHC I (00826)	1 TMD		SVM model	[96]
Integral Membrane proteins (08387, 15517, 09463)	8 (08387) and 9 TMDs		SVM model	[96]
Cell adhesion proteins (06953, 07562, 09392, 06955, 06956)	1 TMD		SVM model	[96]
Receptor kinase (02790, 01554, 01266, 02997, 01555)	1 TMD		SVM model	[96]
G-protein coupled receptors (00045, 01347, 11809, 00678, 03614, 03576, 01628)	7 TMDs		SVM model	[96]
Cell surface receptors (04713)	7 TMDs		SVM model	[96]
p7				
From bioinformatics				
Netrin G1 ligand (12303)	1 TMD		SVM model	[96]
Preadipocyte factor 1 (01446)	1 TMD		SVM model	[96]
Delta like protein (05957)	1 TMD		SVM model	[96]
Notch 4 (01290)	1 TMD		SVM model	[96]
Notch 2 (02606)	1 TMD		SVM model	[96]

cytoplasmic domain consisting of two helices, called 1 and 2. While solid state NMR spectroscopic data give evidence that both helices align parallel to the surface of the lipid membrane [36,37], solution NMR study also suggests a more globular alignment of these helices [34].

2.2. Interaction with TASK

Evidence that Vpu interacts not only with itself and CD4 derives from an investigation in which it has been shown that Vpu also interacts with the TWIK-related acid-sensitive K^+ channel (TASK) and blocks its activity [38] (TWIK stands for tandem pore weakly inward rectifying K channel). Sequence similarity with the first TMD of TASK suggests that Vpu replaces the TASK–TMD and with this impairs the function of TASK. It is found that the consequence of the interaction is a depolarization of the membrane potential [39]. Since it could be shown that membrane depolarization *per se* enhances viral release, it is proposed that the effect of Vpu is, to stimulate budding by mediating depolarization of the plasma membrane. In a more recent study based on cell growth assays

with yeast and a K^+-uptake deficient strain the depolarization effect by modulation of the K-ion concentration is attributed to Vpu to form channels [40].

2.3. Interaction with BST-2

Another membrane protein with which Vpu interacts *via* its TMDs is the interferon induced CD317/tetherin/BST-2 [41–45] (Table 1). BST-2 dimerizes and is incorporated into lipid rafts [46]. BST-2 is known to inhibit viral release by tethering virions to the infected cell surface [41,42]. Vpu recruits the β-TrCP ubiquitin ligase complex which leads to ubiquitination and consequent down-regulation of BST-2. The simultaneous phosphorylation of both serines, Ser-52 and Ser-56 of Vpu, are essential for this mechanism [47]. Important to note is that for the interaction of Vpu with BST-2 the TMDs of both proteins have been found to be relevant (Fig. 1A). It has been shown that with a special Vpu mutant in which the only serine (Ser-23) within the TMD is replaced by a leucine or alanine the down-regulation is not being affected [18,48]. These experiments indicate that the down-regulation is independent of ion channel functionality since this mutation impairs channel activity [16]. Within the TMD of Vpu alanine residues (Ala-18 and -14) are sensitive to the mode of action [18,48] which has sparked the development of a pharmacophore model [49]. First NMR spectroscopic data show evidence of the binding of BST-2 TMD with the alanine rim of Vpu [50]. In another study the triple isoleucine motif, Ile-15, Ile-16, and Ile-17, has been proposed to be an essential site for the interaction with BST-2 [51].

2.4. Interaction with NTB-A

Most recently it has been verified that Vpu is involved in down-modulation of a membrane protein in infected cells relevant for the activation of natural killer (NK) cells [52] (Table 1). NK cells are part of the immune system and able to attack infected cells without being exposed to infected cells ever before. In the case of Vpu, infected T-cells activate the members of the signaling lymphocytic activation molecule (SLM) receptor family. One of these members is the type I integral membrane protein NTB-A, natural killer-T-and-B cell antigen, which acts as a co-activating receptor for NK cells to initiate degranulation. It has been shown that Vpu down-modulates this protein [53]. In contrast to the down modulation of CD4 and BST-2 the phosphorylated cytoplasmic sites (Ser-52 and Ser-56) of Vpu are not essential. The down-modulation is not following the proteasomal pathway. It is therefore speculated that in the presence of Vpu NTB-A is not reaching the cell membrane. Important to note is that the mode of action is similar to BST-2, as much as the TMD of NTB-A is responsible for it (Fig. 1A). Experiments with a scrambled TMD sequence of Vpu, similar to the one used before by others [7], down-modulation is not observed.

2.5. Interaction with CD74

The two major histocompatibility complexes (MHC) I [54] and the MHC II invariant chain (Ii), also called CD74, and a precursor of a peptide presenting MHC II (pMCH II) [55], are reported to be down-regulated by the involvement of Vpu. MHC proteins, class I proteins, are essential to report the host-own character of the cell and are also essential for presenting peptide antigens to initiate CD8 + T cells for defense [56]. Interaction of the cytoplasmic domain of Vpu with both MHC proteins is proposed to be necessary.

2.6. Interaction with TPR

A member of the tetratricopeptide repeat (TPR) protein family has also been reported to interact with Vpu [57]. The 41 kDa protein contains four copies of a 34-amino acid TPR motif which interacts with Vpu. The protein is named as Vpu-binding protein (UBP). Its role is to interfere with the interaction of UBP with HIV-1 Gag protein. A site of Vpu-UBP interaction has not yet been identified.

2.7. Motifs for protein–protein interaction in Vpu

In a bioinformatics approach short eukaryotic linear motifs (ELMs) relevant for protein–protein interactions between host proteins are proposed to be present in HIV-1 proteins [58]. Among the few ELMs suggested for Vpu, one motif, TRG_LysEnd_APsACLL_1 (pattern: [DERQ]…L[LVI], http://elm.eu.org/), is a common motif in type I integral membrane proteins and found in their membrane–water interfacial regions. It seems to be evident, that Vpu harbors motifs common for protein–protein interaction and thus, a considerable role of Vpu's should comprise indeed the steering of the host cell *via* this type of interaction (Fig. 2).

At this stage of research the mode of action, as to interact with membrane proteins of the host to steer cellular function and with it the survival of the virus, is well established. In as much ion channel function is essential for the role of viral release enhancement is still under debate.

3. E5 of human papillomavirus 16

Human papilloma virus (HPV) plays an important role in the pathogenesis of warts, and cancers of the skin, cervical [59] and anogenital tract [60,61]. In the case of cervical carcinoma subtype HPV-16 belongs to the 'high risk' subgroup which is highly associated with the development of cervical cancer induced by infection with the virus. The genome of HPV-16 encodes a number of proteins which are expressed in the early stage of viral infection and found to be oncogenic, E6, E7 [62,63] and E5 [64,65]. In contrast to E6 and E7, expression of E5 is often suppressed later when the genome of HPV-16 is integrated into the host cell genome during progression to malignant disease [66,67]. Therefore E5 is considered to be essential in the early stage of infection [68]. There is increasing evidence that the three proteins have synergistic effects for some mechanisms within the viral infectivity cycle [69]

E5 is an 83 amino acid polytopic membrane protein harboring three TMDs and located at the Golgi apparatus, endoplasmic reticulum and nuclear membrane (Fig. 1B, left). A shorter 44 amino acid bitopic E5 protein of bovine papilloma virus (BPV) with its TMD at the N terminal side shares 27% sequence homology with this protein as well as a leucine rich motif at both of their N termini [70,71]. Analysis of the sequence of HPV-16 E5 sequence using the Kyte–Doolittle scale of hydrophobicity identifies the three putative TMDs [72]. E5 dimerizes on the bases of hydrophobic interactions of its TMDs [73], possibly in a concentration dependent manner [74]. Deletion studies could not single out one TMD responsible for dimerization [73]. Dimerization is not driven by the cysteines present at the N terminal side and near the C terminus since reducing agents do not affect the capability of E5 to form dimers [72]. Experiments with epitope tagged E5 at both of its ends reveal that its N-terminal side is located toward the ER while its C-terminus is exposed to the cytoplasm [74]. FTIR spectroscopic investigations of short peptide constructs, which include the putative TMDs, suggest TMDs to be helical [75]. The peptides have been derived from solid phase peptide synthesis (SPPS) and dissolved in buffer/trifluoroethanol (TFE) mixture (90 μl/10 μl). In a similar way, CD-spectroscopic analysis has been done with peptide constructs including the putative TMDs derived from SPPS dissolved in various mixtures of buffer/TFE [76]. Besides spectra recordings in different solvent mixtures, also a series of mixtures of the individual peptides have been measured. The data suggest a large β-sheet content for the first and third TMDs and irregular structure for the second TMD. Due to the large content of leucines in the first and third TMDs the authors relate the structural motif to leucine-rich repeat (LRR) proteins which typically adopt a large content of β-fold. Secondary structure prediction programs for both, helical and β-sheet structure propose a helical motif for the three TMDs (Mahato and Fischer unpublished results). Despite the application of almost membrane like conditions using TFE [76] it is necessary to include more thorough

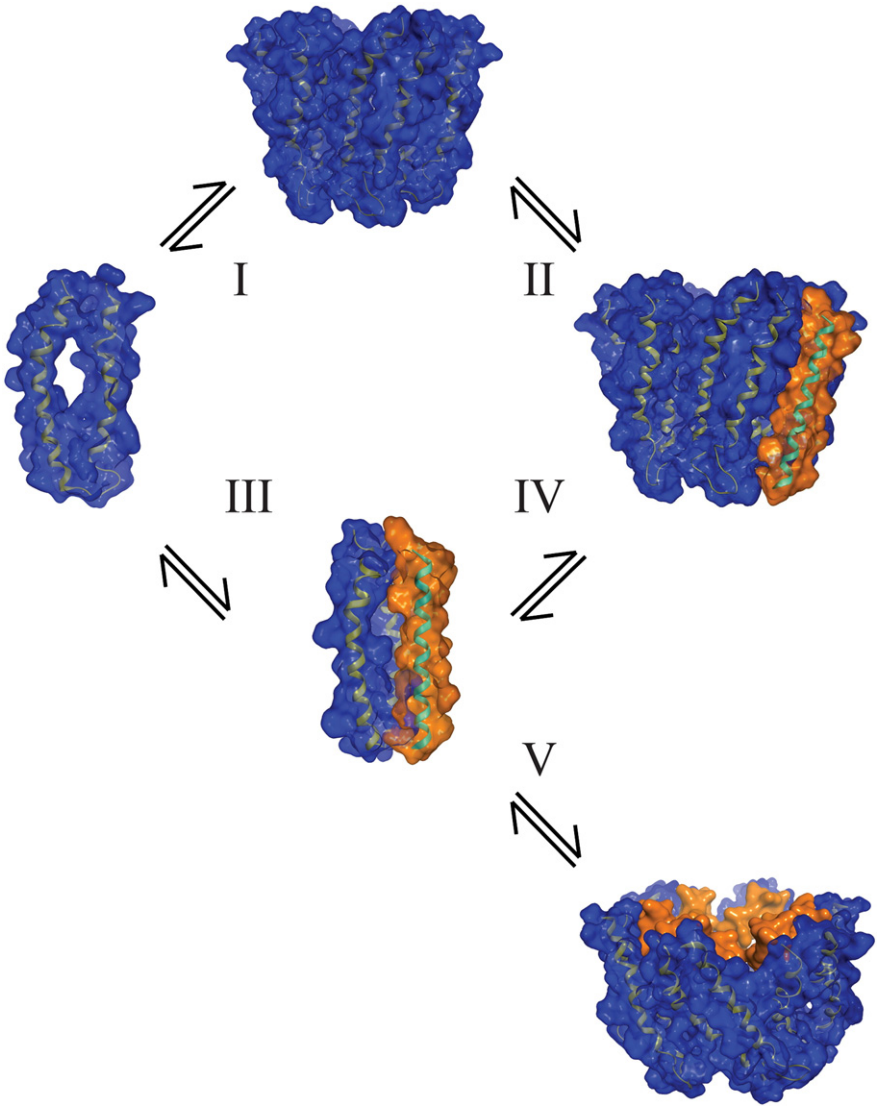

Fig. 2. Putative reaction schemes of the monomeric channel forming proteins (blue, surface mode of MOE suite with highlighted backbone) into homo- or heterooligomers with membrane proteins of the host (orange). It is assumed that there is equilibrium between the monomer and a homooligomeric- or bundle-like form (I). The bundle can consequently interact with the transmembrane domains (TMDs) with the host (II). Another pathway is suggested to allow the monomer to interact with the host protein first (III). In a consequent step the monomer-host assembly either integrats together with other viral monomers into a viral bundle (IV) or forms an oligomeric monomer-host assembly (V).

lipid-membrane like conditions in future experiments to unravel the discrepancy in the experimental results reported above.

Recently, it has been proposed that E5 exhibits channel activity using fluorescent dye release assays with liposomes [11]. Channel activity has been shown to be sensitive to pH, which could possibly be related to the histidines present in TMD3. The pore size is proposed to be in a range of 1.2 to 2.8 nm using fluorescein isothiocyanate conjugated dextrans of various molecular masses in the liposome assay. Based on SDS PAGE analysis the oligomeric state of HPV-16 E5 is suggested to be hexameric and modeled accordingly with a monomeric unit consisting of three TMDs (Fig. 1B, right). It could be shown that rimantadine and a series of novel compounds (named MV003 and MV006) block channel activity.

Similar to Vpu, E5 exerts multiple functions ([77,78] see for review [71,79]).

The following host proteins are identified to be involved in protein–protein interaction with E5 (Table 1).

3.1. Interaction with 16 kDa protein

Similar to E5 of BPV, HPV-16 E5 interacts with the 16-kDa pore-forming protein component of the vacuolar H^+-ATPase [70,80]. Mutant studies with E5 of BPV show that glutamine residue at position 17 is essential for the interaction [80]. In the case of HPV-16 E5 it seems that the hydrophobicity of TMD2 is important for the interaction [81] as well as TMD3 [82]. With a series of E5 mutants (Y63F/Y68F, H75N, H77Q, H75F/H78Q) it could be shown that the tyrosines and histidines are comprising a binding motif of E5 with 16 kDa protein.

3.2. Interaction with HLA-I

Down-regulation of the human leukocyte antigens (HLA) class I is observed in HPV-16 E5 expressing cell lines [83], which parallels the same activity of E5 BPV [84]. It is proposed that the first hydrophobic domain of E5 is interacting with the heavy chain of HLA-I [85]. In another study it is shown that HPV-16 E5 and HLA-I co-precipitates together with calnexin and identifies the first TMD of E5 to be involved in the association [86]. Mutating three leucine residues to proline, aspartate and arginine (triple mutant L18P/L23D/L28R) within TMD1 abrogates interaction with calnexin and down-regulation of HLA-I.

In case of interaction of E5 with HLA-I and the 16 kDa protein via the first and the last two TMDs, respectively, a simultaneous binding of a single E5 protein with both proteins is proposed [85].

3.3. Interaction with growth factors EGFR and ErB4

E5 protein of HPV-6 associates with the epidermal growth factor receptor (EGFR), platelet-derived growth factor (PDGF) and ErbB2, but HPV-16 E5 does not [87]. However, in the presence of HPV-16 E5 recycling of EGFR to the cell surface is increased [88]. The C terminal part of E5 including the last 5 amino acids is important for binding and initiating over-activation of EGFR [82].

E5 interacts with ErbB4 a member of the tyrosine kinase growth factor family ErbB [78]. The TMD of ErbB4 and extracellular domains of ErbB4 are necessary for binding. This implies that possibly the TMDs of E5 are involved in the binding.

3.4. Promoting perinuclear fusion of membrane koilocytosis

Membrane fusion regulating complex calpactin I is a target of E5 [69,89]. Calpactin I is a heterodimer of two annexin A2 and p11 subunits. Binding *via* its 20 C-terminal amino acids to both subunits of calpactin I, E5 promotes perinuclear redistribution of calpactin I with the consequence of formation of koilocytotic vacuoles by perinuclear membrane fusion. The 20 amino acids of E5 fully include its TMD3. Deletion of only 10 C-terminal amino acids leaves the protein with half of its activity [69].

3.5. ER transport protein, gap junctions and others

For regulating Bap31, a polytopic B-cell receptor-associated (Bap) membrane protein involved in the export of transmembrane proteins from the ER [90], the last 10 amino acids of the C-terminus of E5 are found to be essential [91]. Similarly, the last 10 amino acids of E5 are also relevant to associate with karyopherin β3, a member of the nuclear import receptor family [92]. Interference with the stress response of cells in the presence of E5 indicates that especially residues in TMD3 are highly relevant such as His-77 and Tyr-63 [93].

Furthermore, E5 is also involved in down-regulation of connexin 43 [94] and alteration of lipid composition in infected cells [95].

It has also been predicted that E5 interacts with a series of host factors using bioinformatics approaches based on support vector machine (SVM) models [96] (Table 1).

4. p7 of hepatitis C virus

The p7 HCV is expressed as part of a large polyprotein which is cleaved by cellular and viral proteases into ten cleavage products ([97,98], for a recent review see [99]). It is a 63 residue, 6-7 kDa polytopic membrane protein with two TMDs linked via a very short loop (Fig. 1C). Its role is expected to be active during entry into the host cell [100]. Furthermore it is also essential for particle assembly [101,102]. The two TMDs are connected *via* a short hydrophilic segment; both ends of the TMDs are found to point into the ER lumen [103]. Channel characteristics identify p7 to be a weak cation selective channel [104–106] which is sensitive to the presence of Cu^{2+} [107] and small ligands [108].

The oligomerization state of p7 is dependent on the protein:detergent ratio ($C_{12}E_8$) when using sedimentation experiments [105]. At lower ratio a hexameric assembly is proposed which can shift to slightly higher numbers at higher ratios. These findings are supported by cryo EM pictures of assembled p7 proteins in either hexameric or heptameric form [109–111]. Structural information is currently still on the bases of NMR spectroscopic investigations suggesting that the overall two TMDs can each be subdivided into two small helical sections [105,112,113]. Computational models have been generated supporting the role of histidines as pore lining residues [114,115].

Interactions of p7 with host factors are proposed using the same bioinformatics approaches as mentioned above [96], as well as a genetic screening approach [116] (Table 1). Some of the host proteins are identified as globular proteins [116]. The proposed membrane proteins are as follows [96]:

- Netrin-G1 ligand (protein binding), which stimulates the growth of embryonic thalamic axons by binding of netrin-G1 [117] (single TMD)
- Preadipocyte factor 1 (growth factor), involved in cell differentiation of adipocytes (reviewed in [118] (single TMD)
- Notches 4 and 2 (cell surface receptors) involved in cell signaling [119] (single TMD).

Experimental evidence of interactions has not yet been reported but is proposed to play a major role in future drug therapy [120].

5. Conclusion

The lipid membrane is a unique location for proteins. Similar to the cytoplasm of the cell, it seems to be used for protein–protein communication. The hydrophobic nature of the membrane restrains the application of amino acids being used. Yet, a complicated communication network in respect of specificity of interactions is established. The proteins presented in this review are found to homo-oligomerize and to form dimers and possibly also hetero-oligomers with host factors (Fig. 2). It is fascinating to learn about the directionality of the interactions between these proteins and specifically the interaction between their TMDs. The consequences will be highly important when it comes to manufacture complex membrane proteins or to develop novel concepts in antiviral therapy.

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