VIROPORINS

Maria Eugenia Gonzalez[1]* and Luis Carrasco[2]

[1]Unidad de Expresión Viral, Centro Nacional de Microbiología. Instituto de Salud Carlos III. Carretera de Majadahonda-Pozuelo Km 2. 28220 Majadahonda, Madrid, Spain

[2]Centro de Biología Molecular Severo Ochoa. Facultad de Ciencias, Universidad Autónoma. Cantoblanco, 28049 Madrid, Spain

* Corresponding author. Phone: (34) 91 822 3698. Fax (34) 91 509 7919. e-mail: megonzalez@isciii.es

Key words: Animal viruses; Membrane permeability; Membrane pores; Channel ions; Virus budding

SUMMARY

Viroporins are a group of proteins that participate in several viral functions, including the promotion of release of viral particles from cells. These proteins also affect cellular functions including the cell vesicle system, glycoprotein trafficking and membrane permeability. Viroporins are

not essential for the replication of viruses, but their presence enhances virus growth. Comprising some 60-120 amino acids, viroporins have a hydrophobic transmembrane domain that interacts with and expands the lipid bilayer. Some viroporins also contain other motifs such as basic amino acid residues or a domain rich in aromatic amino acids that confers the protein the ability to interact with the interfacial lipid bilayer. Viroporin oligomerization gives rise to hydrophilic pores at the membranes of virus infected cells. As the list of known viroporins steadily grows, recent research efforts focus on deciphering the actions of the viroporins poliovirus 2B, alphavirus 6K, HIV-1 Vpu and influenza virus M2. All these proteins can enhance the passage of ions and small molecules through membranes depending on their concentration gradient. Future work will lengthen the list of viroporins and will provide a deeper understanding of their mechanisms of action.

1. INTRODUCTION

Viruses inflict a number of injuries as they infect susceptible cells. Some of these injuries affect cell membranes, such that the plasma membrane and intracellular vesicle system become modified. A typical feature observed during the action of most animal viruses is enhanced membrane permeability [1]. Several viral gene products could be responsible for these changes, including proteases [2,3], glycoproteins [4-7] and viroporins [1].

2. DEFINITIONS AND EARLY WORK

Viroporins are small, highly hydrophobic, virus-encoded proteins that interact with membranes modifying the cell's permeability to ions or other small molecules. The name viroporin

was first proposed when it was found that several virus proteins shared common characteristics [8]. This concept was later revised in depth [1].

That viroporins existed was predicted many years ago, when enhanced membrane permeability was noted in several virus-cell systems [9,10]. Two different modes of membrane leakiness have been distinguished according to the time of infection. Early membrane modification linked to virus entry requires no gene expression since it is the virion's components that mediate these alterations [11,12]. As infection progresses, several viral products can affect cell membranes. Amongst these products, viroporins are responsible, at least in part, for membrane leakiness occurring late in infection.

3. GENERAL FEATURES OF VIROPORIN STRUCTURE

Typically, viroporins are comprised of some 60-120 amino acids (Table 1). They contain a highly hydrophobic domain able to form an amphipathic α-helix. The insertion of these proteins into membranes followed by their oligomerization creates a typical hydrophilic pore with hydrophobic amino acid residues facing the phospholipid bilayer and hydrophilic resides forming part of the pore [13-16]. Apart from this domain, there are several additional features of viroporin structure that warrant mentioning. Hence, some viroporins contain an additional hydrophobic region that interacts with membranes. This may disturb the organization of the lipid bilayer. The viroporin may also contain a stretch of basic amino acids that acts like a detergent (Figure 1). All these features contribute to membrane destabilization.

Recently, another domain has been detected in some viroporins that has the capacity to interact with membranes. This domain is rich in aromatic amino acids and is usually inserted in the

interfacial phase of the phospholipid bilayer [17]. This type of interaction also leads to membrane destabilization, thus enhancing membrane permeability.

4. VIRUS GLYCOPROTEINS THAT MODIFY MEMBRANE PERMEABILITY

Several types of virus proteins are able to modify membrane permeability. *Sensu stricto*, this property defines the activity of a viroporin. However, apart from small hydrophobic viral proteins, there are other virus products that promote membrane permeabilization. This is true of a number of virus glycoproteins that are known to increase cell membrane permeability [4,6,7,18-22]. The architecture of some viral glycoproteins is such that upon oligomerization, it may conform a physical pore. In principle, physical viral glycoprotein pores could be formed by the fusion of peptides acting in conjunction with transmembrane domains. The fusion peptides would create a pore in the cell membranes upon insertion, while the TM domain would form a pore in the virion membrane. Moreover, domains adjacent to the transmembrane region could have motifs designed to destabilize membrane structure. Thus, in viruses that lack the typical viroporin, its function could be replaced by these pore-forming glycoproteins, while for other viruses viroporin activity may be redundant [23,24]. In this last case, pore formation may be achieved by both viral glycoproteins and viroporins. We would like to propose the possibility that pore-forming glycoproteins play a key role mainly during virus entry and in some cases also during virus budding, while viroporins come into action when viruses need to exit the cell. In conclusion, some viruses could have developed glycoprotein structures capable of destabilizing membranes that totally or partially replace viroporin function. Viroporin activity might be fully replaced in viruses, such as HIV-2, that lack typical viroporins.

Besides these glycoproteins, there are currently about a dozen proteins that qualify as typical viroporins (Table 1). However, most work on the structure-function of viroporins has concentrated on four of these proteins.

5. PICORNAVIRUS 2B

Picornavirus proteins arise from a large polyprotein precursor that is cleaved by viral proteases [25]. The picornavirus 2B gene codes for a protein of about 100 residues, depending on the virus species considered (Table 1). The 2B product contains two hydrophobic regions (Figure 1). At least one of these regions spans the membrane by means of an amphipathic helix. Using the two-hybrid system, it was determined that there are 2B homointeractions [26,27]. The formation of 2B homooligomers has been confirmed by an elegant approach involving fluorescence resonance energy transfer microscopy [28]. In fact, it seems that most of the 2B protein located at membranes oligomerizes as dimers and tetramers [14]. It was initially shown that both poliovirus 2B and 3A proteins had a permeabilizing effect on cells, though attention soon turned to just the 2B protein [29,30]. Notably, the 2BC precursor is the most permeabilizing viral protein, perhaps due to the conformation adopted by 2B in this precursor, or to differences in the subcellular location of 2B and 2BC, or to an intrinsic, still unveiled activity of 2BC [31,32]. Certainly 2C alone is devoid of permeabilizing capacity in cells. All three proteins, 2B, 2BC and 3A, interact with membranes and are found to mostly localize at intracellular membranes [33-36]. The subcellular location of each of these proteins when individually expressed may be altered by coexpression of combinations of the three [37].

Not only do 2B, 2BC and 3A enhance membrane permeabilization, but they are also able to promote intracellular membrane remodelling [33-35,38], leading to disruption of the vesicle system

and glycoprotein trafficking, including the expression of receptors on the cell surface [30,39]. Unfortunately, poliovirus lacking the 2B gene is not viable, since intracellular membrane remodelling is a prerequisite for viral genome replication [35]. Poliovirus and coxsackievirus 2B and 2BC variants have been analyzed in detail [32,36].

Using liposomes, it has been possible to estimate the size of the pore formed by 2B at 6 Å in diameter [14]. This pore size allows the diffusion of molecules of MW under 1.000 Da. Along with pore size, the degree of 2B oligomerization might be conditioned by the lipid composition of the membranes. The formation of this hydrophilic pore by poliovirus 2B is a hallmark in our knowledge of viroporins. Besides ions, small molecules can pass through these pores; a behaviour that is very similar to that noted in animal virus-infected cells [35].

6. ALPHAVIRUS 6K

Alphavirus 6K is synthesized as part of a larger precursor that is proteolytically cleaved [40]. Immediately after synthesis and prior to exit from the endoplasmic reticulum, the 6K protein could form a complex with E1 and p62 (precursors of E2 and E3), persisting as such during transport to the cell surface [41]. Mature glycoproteins become incorporated in the new virions, whereas 6K is mostly excluded [41,42]. The 6K protein crosses the membrane once and only its N terminus is located in the ER lumen [15]. 6K has several roles: it provides sites for protease cleavage, it may participate in viral glycoprotein trafficking and, finally, 6K enhances membrane permeability [43-45]. Alphavirus 6Ks are composed of about 60 residues (Table 1) including a long stretch of very hydrophobic amino acids that confer the capacity to interact with membranes [40]. Some amino acids are acylated, increasing their ability to persist on the membranes [40]. Recently, a domain able to become partitioned among membranes was identified in Sindbis virus 6K [17]. This domain is required to enhance membrane permeability. The individual expression of 6K promotes membrane

permeabilization [17,45]. These studies have been complemented by the analysis of 6Kdeficient togavirus variants. The 6K protein is not essential for virus particle formation, nor for early viral infection steps such as the binding, uptake and uncoating of the infecting virus along with the formation of early nonstructural virus protein [46,47]. Total deletion of the 6K gene still permits virus replication, although virus yields are diminished [48,49]. 6K-defficient viruses retain their capacity for glycoprotein processing; virus budding being the most affected step of virus replication [49,17]. It has been suggested that 6K might exert its actions on glycoprotein packing and on its interaction with membrane lipid [50]. Interestingly, Sindbis viruses that lack 6K are compensated, at least in part, by the synthesis of HIV-1 Vpu [51]. The recent discovery of a cation-selective ion channel activity of 6K proteins from BFV and RRV when inserted into planar lipid bilayers [15], has opened new routes for further exploring the mechanisms of action of alphavirus 6K protein.

7. HIV-1 Vpu

HIV-1 Vpu is an oligomeric, type I transmembrane phosphoprotein [52-54]. Vpu is translated from a bicistronic mRNA that also encodes the envelope glycoprotein. The *vpu* gene is unique to HIV-1, it is not encoded by HIV-2, nor by simian lentivirus with the exception of SIV_{cpz}. However, the envelope glycoprotein of lentivirus may display Vpu-like activity in the absence of Vpu protein [23]. The HIV-1 Vpu protein contains 81 amino acid residues distributed along an N-terminal hydrophobic region of 27 amino acid residues and a C-terminal hydrophilic region, connected by a short stretch of basic amino acid residues (Figure 1). In the HIV-1 life cycle, Vpu has at least two roles that correspond to two domains.

The N-terminal transmembrane segment is critical for Vpu enhancement of virus particle release from infected cells [55]. The C-terminal cytoplasmic domain is required for CD4 degradation

in host cells [56]. Most Vpu protein localizes at the ER and the Golgi apparatus; a small amount of protein, which is nevertheless excluded from the virus particles, localizing at the cytoplasmic membrane [57,58]. According to this cellular location, Vpu induces modifications in compartments in the secretory pathway and the cell membrane. Thus, Vpu impairs normal trafficking of membrane proteins other than CD4, such as MHC-I and alphavirus glycoproteins [51,59]. In addition, Vpu disrupts cell membrane integrity inducing permeability to small molecules upon expression in *Escherichia coli* and in mammalian cells [60]. According to secondary structure predictions, Vpu and lysin from red abalone (*Haliotis rufescens*) have been reported to be similar [61].

Several lines of evidence reflect the ion channel activity of Vpu. Purified Vpu forms ion channels with a slight preference for cations in planar lipid bilayers [62]. When expressed in *Xenopus* oocytes, Vpu increases cation selective membrane conductance [63]. Further, it has been recently shown that amiloride derivatives block Vpu ion channel activity [64]. However, the Vpu protein and its mRNA are both unstable in oocytes. There is some dispute over whether Vpu acts as an ion channel at the cell surface or only as an intracellular ion channel [65,66].

The Vpu protein contains three helical units, one of which is an amphipathic transmembrane helix. Synthetic Vpu transmembrane helices have been shown to selfassemble in a lipid bilayer to form channels [63]. Molecular dynamic simulations of ion channels formed by bundles of Vpu transmembrane helices suggest the most likely channel assembly is a pentamer, but higher or lower order oligomers may also be formed [16]. Further studies based on FTIR spectroscopy combined with a global molecular dynamics search protocol, indicate that tryptophans may occlude the pore of the pentameric bundle of helices by forming a stable assembly, whereas the gating mechanism might consist of conformational changes that take place in the transmembrane peptide [67]. When extended transmembrane segments of Vpu were used, arginines (Arg 31) pointed into the pore forming a positive charged ring that could act as a putative selectivity filter [68].

Despite Vpu's multifunctional role in the virus life cycle, HIV-1 carrying a truncated *vpu* gene is still able to replicate. The steps affected in *vpu*-deficient variants are the correct assembly and exit of virus particles [69]. A large proportion of mutant particles remains attached to the cell surface, being the size and shape of these progeny virions also altered. In good agreement with these findings, *vpu* expression is able to correct defects in Sindbis viruses lacking the 6K gene [51].

8. INFLUENZA VIRUS M2

The M2 protein is encoded by a small genomic influenza virus RNA fragment [65]. This RNA fragment also codes for another protein known as M1. M2 or M1 synthesis occurs on different mRNAs, generated by differential splicing. M2 is a type III integral membrane phosphoprotein made up of 96 amino acids, which can be divided into three regions: an extracellular 23-residue fragment, a 19-amino acid transmembrane domain and a final 54 residues comprising the cytoplasmic tail [65]. The M2 protein forms homooligomers whose active state is a tetramer. A break-through in elucidating M2 functioning emerged from studies on its individual expression in *Xenopus laevis* oocytes [70]. M2 synthesis in this system leads to increased permeability towards ions, with the consequent decrease in membrane potential. The current M2 activity model indicates this protein may act at two different stages during influenza virus infection (See reviews:[65,71]). M2 allows the entry of protons into virions promoting virus uncoating in endosomes [72]. In addition, the ion channel activity of M2 might lead to a pH balance in influenza virus-infected cells, between that of the acid lumen of the TGN and the pH of the cytoplasm [72-74]. Detailed analyses of the structure and function of M2 as an ion channel are reviewed in this issue.

M2 also has effects on glycoprotein processing and trafficking. The M2 proteins impairs the correct glycosilation of the viral glycoprotein and slows HA delivery to the plasma membrane [74,75]. In addition, M2 reduces the apical secretion of cellular proteins in MDCK cells [76,77].

The antiviral compound amantadine, which blocks the entry of all strains of influenza virus [78], inhibits M2 [73] and also hinders the proper budding of virus particles [79].

In direct contrast to the above model of M2 functioning is the finding that this protein increases membrane permeability to small molecules, as occurs with other viroporins [80]. Moreover, influenza virus entry does not occur at low pH when the pH gradient is destroyed [12,81]. An M2-deficient variant is able to undergo multiple cycles of replication in cultured cells, further suggesting that M2 is not essential for virus entry [82]. In this M2-deficient variant, virus production is decreased with respect to wild type influenza virus [82,83], consistent with observations in other animal viruses lacking the viroporin gene [49,69]. The possibility that M2 activity is differentially required for infectivity according to virus strain has been argued [83].

9. OTHER VIROPORINS

As well as the viroporins described above, there are other examples that have received less attention. The structural features of a number of animal virus proteins fits in well with the typical viroporin structure. In some cases their membrane permeabilization capacity has also been proven. This is true of the SH protein of respiratory syncytial virus and the p10 protein of avian reovirus. [84,85]. Another recent example is the small hydrophobic protein, p7, that resembles alphavirus 6K, encoded by species of the Flaviviridae family. Human hepatitis C virus (HCV) p7 has been found to be associated with secretory pathway compartments, a small fraction also being located at the plasma membrane [86]. HCV p7 forms hexamers and functions as a calcium channel in black lipid membranes

[87]. Further, the p7 protein of bovine viral diarrhea virus (BVDV) facilitates virus release from the plasma membrane [88]. A nice example of a potassium channel protein encoded by algae virus corresponds to Kcv protein from *Paramecium bursaria* chlorella virus (PBCV-1) [89,90]. The Kcv protein contains two membrane-spanning domains linked by a strech of 44 amino acids. Kcv fused to GFP has a preferential localization at the endoplasmic reticulum and much less at the plasma membrane [91].

Other proteins proposed as viroporins because of their structural features need further investigating to establish their membrane permeabilization capacity. One promising candidate is the 10.6 KDa polypeptide encoded by the alpha 1 ORF gene in bovine ephemeral fever rhabdovirus (BEFV). This protein has the hydrophobic and highly basic regions characteristic of a viroporin, but its functional role remains to be explored [92]. Recently, a new vaccinia virus gene, A14.5L ORF, has been predicted to encode a hydrophobic protein comprised of 53 amino acids. Although the A14.5L product is not essential for virus replication in tissue cultured cells, deletion of this gene reduces the virulence of vaccinia virus in mice [93].

Several phage proteins also show the typical viroporin structure [94]. These proteins are able to modify membranes and can open large pores in bacterial cells. Because of this capacity, they have been denoted holins. To our knowledge, no viroporins have been described in plant viruses. The analysis of plant virus genomes could, nonetheless, uncover their existence. Indeed, this could be the case of the small hydrophobic protein (p6) of Closteroviruses, which is known to be involved in virus movement from cell to cell [95].

10. FUTURE PROSPECTS

Despite significant developments in the field of viroporins since our last review on this topic, we foresee that this decade will bring further insight into this fascinating group of viral proteins. Several questions related to the functions of viroporins await clarification and future research lines will no doubt attempt to address questions such as:

Subcellular localization of viroporins

Immunolocalization studies performed on virus infected cells indicate that most of the viroporin is located intracellularly, while little or none is detected at the plasma membrane. This might suggest that viroporin acts by releasing a signal from an intracellular compartment to the plasma membrane, where viroporin activity is detected. Alternatively, perhaps only the small amounts of viroporin observed at the plasma membrane can account for the enhanced membrane permeability. By retaining some viroporins at intracellular compartments, it has been possible to block their membrane permeability enhancing capacity. This suggests the protein needs to reach the plasma membrane to act. Finally, it should be kept in mind that the destiny of a viroporin could be determined or modulated by the synthesis of other viral components [37].

Interaction of viroporins with other molecules

Another question of interest would be to determine if viroporins interact with other viral or cellular proteins. If this were the case, viroporin trafficking through the vesicle system could be regulated, as could the formation of the actual pore. For instance, if a viroporin molecule interacts with a given viral glycoprotein, dissociation of this interaction could promote viroporin oligomerization and pore formation [41,96].

Ion channels or hydrophilic pores

An essential point that needs to be established is whether viroporins form pores allowing the passage of different ions and small molecules or if their architecture itself corresponds to specific ion-channels with a controlled gating mechanism [71]. In general, ion channels are selective for a given ion and do not permit the passage of other ions or molecules. Moreover, the gating of these

channels is regulated. Phenomena observed in virus-infected cells or in systems that express some of these viroporins would appear to indicate the presence of pores at the plasma membrane (Figure 2).

Which is their exact mode of action?

The key question is to understand, in molecular terms, how a protein that forms pores can enhance virus budding from cells. A possibility is that the dissipation of ionic gradients in regions of virus assembly promotes virion exit from cells [97].

Are there different viroporin subfamilies?

The discovery of new viroporins and subsequent analysis of their activity will provide information on the molecular uniformity of this group of proteins. Present knowledge suggests there are different viroporin subfamilies, since their structure and function differs when analyzed in detail. This question would need to be addressed by comparative studies on several viroporins.

Viroporins and antiviral chemotherapy

The fact that some compounds such as amiloride derivatives or amantadine are able to block Vpu, M2 and p7 suggests the possibility of finding additional selective agents towards viroporin function. Inhibiting viroporin activity would lead to diminished virus production. This alone might be sufficient for the immune system to eradicate the virus infection. Notwithstanding, regardless of their therapeutic potential or lack of anti-viral activity, new anti-viroporin compounds will be extremely useful for evaluating the mode of action of these proteins in cell culture systems.

ACKNOWLEDGEMENTS

This work was supported by the Comunidad Autonoma de Madrid (082-0024/2000), Instituto de Salud Carlos III (01/0042) and the DGICYT (PM99-0002). The authors also acknowledge the institutional grant awarded to the Centro de Biología Molecular

"Severo Ochoa" by the Fundación Ramón Areces.

REFERENCES

1 Carrasco, L. (1995) Adv Virus Res 45, 61-112.

2 Chang, Y. S., Liao, C. L., Tsao, C. H., Chen, M. C., Liu, C. I., Chen, L. K. and Lin, Y. L. (1999) J.Virol. 73, 6257-6264.

3 Blanco, R., Carrasco, L. and Ventoso, I. (2003) J Biol.Chem. 278, 1086-1093.

4 Newton, K., Meyer, J. C., Bellamy, A. R. and Taylor, J. A. (1997) J.Virol. 71, 9458-9465.

5 Tian, P., Estes, M. K., Hu, Y., Ball, J. M., Zeng, C. Q. and Schilling, W. P. (1995) J Virol 69, 5763-5772.

6 Arroyo, J., Boceta, M., Gonzalez, M. E., Michel, M. and Carrasco, L. (1995) J.Virol. 69, 4095-4102.

7 Ciccaglione, A. R., Marcantonio, C., Costantino, A., Equestre, M., Geraci, A. and Rapicetta, M. (1998) Virology 250, 1-8.

8 Carrasco, L., Perez, L., Irurzun, A., Martinez-Abarca, F., Rodriguez, P., Guinea, R., Castrillo, J. L., Sanz, M. A. and Ayala, M. J. (1993) in: Regulation of gene expression in animal viruses (Carrasco, L., Sonenberg, N. and Wimmer, E. Eds.) pp 283-303, Plenum Publ. Corp., New York.

9 Carrasco, L. (1978) Nature 272, 694-9.

10 Carrasco, L. (1979) in: Antiviral mechanisms for the control of neoplasia (Chandra, P.) pp 623-631, Plenum Press, New York.

11 Fernandez-Puentes, C., Carrasco, L. (1980) Cell 20, 769-75.

12 Carrasco, L. (1994) FEBS Lett. 350, 151-154.

13 Pinto, L. H., Dieckmann, G. R., Gandhi, C. S., Papworth, C. G., Braman, J., Shaughnessy, M. A., Lear, J. D., Lamb, R. A. and DeGrado, W. F. (1997) Proc.Natl.Acad.Sci.U.S.A 94, 11301-11306.

14 Agirre, A., Barco, A., Carrasco, L. and Nieva, J. L. (2002) J.Biol.Chem. 277, 40434-40441.

15 Melton, J. V., Ewart, G. D., Weir, R. C., Board, P. G., Lee, E. and Gage, P. W. (2002) J.Biol.Chem. 277, 46923-46931.

16 Grice, A. L., Kerr, I. D. and Sansom, M. S. (1997) FEBS Lett 405, 299-304.

17 Sanz, M. A., Madan, V., Carrasco, L. and Nieva, J. L. (2003) J.Biol.Chem. 278, 2051-2057.

18 Chernomordik, L., Chanturiya, A. N., Suss-Toby, E., Nora, E. and Zimmerberg, J. (1994) J Virol 68, 7115-7123.

19 Comardelle, A. M., Norris, C. H., Plymale, D. R., Gatti, P. J., Choi, B., Fermin, C. D., Haislip, A. M., Tencza, S. B., Mietzner, T. A., Montelaro, R. C. and Garry, R. F. (1997) AIDS Res.Hum.Retroviruses 13, 1525-1532.

20 Dong, Y., Zeng, C. Q., Ball, J. M., Estes, M. K. and Morris, A. P. (1997) Proc.Natl.Acad.Sci.U.S.A 94, 3960-3965.

21 Charpilienne, A., Abad, M. J., Michelangeli, F., Alvarado, F., Vasseur, M., Cohen, J. and Ruiz, M. C. (1997) J Gen.Virol 78, 1367-1371.

22 Sanderson, C. M., Parkinson, J. E., Hollinshead, M. and Smith, G. L. (1996) J Virol 70, 905-914.

23 Bour, S., Strebel, K. (1996) J Virol 70, 8285-8300.

24 Suarez, T., Gallaher, W. R., Agirre, A., Goni, F. M. and Nieva, J. L. (2000) J Virol 74, 8038-8047.

25 Leong, L. E. C., Cornell, C. T. and Semler, B. L. (2002) in: Molecular biology of picornavirus (Semler, B. L., Wimmer, E. Eds.) pp 187-197, ASM Press,

Washington.

26 Cuconati, A., Xiang, W., Lahser, F., Pfister, T. and Wimmer, E. (1998) J.Virol. 72, 1297-1307.

27 De Jong, A. S., Schrama, I. W., Willems, P. H., Galama, J. M., Melchers, W. J. and van Kuppeveld, F. J. (2002) J.Gen.Virol. 83, 783-793.

28 van Kuppeveld, F. J., Melchers, W. J., Willems, P. H. and Gadella, T. W., Jr. (2002) J Virol 76, 9446-9456.

29 Lama, J., Carrasco, L. (1992) J.Biol.Chem. 267, 15932-15937.

30 Doedens, J. R., Kirkegaard, K. (1995) EMBO J. 14, 894-907.

31 Aldabe, R., Barco, A. and Carrasco, L. (1996) J.Biol.Chem. 271, 23134-23137.

32 Barco, A., Carrasco, L. (1998) J Virol 72, 3560-3570.

33 Cho, M. W., Teterina, N., Egger, D., Bienz, K. and Ehrenfeld, E. (1994) Virology 202, 129-145.

34 Egger, D., Gosert, R. and Bienz, K. (2002) in: Molecular biology of picornavirus (Semler, B. L., Wimmer, E. Eds.) pp 247-253, ASM Press, Washington.

35 Carrasco, L., Guinea, R., Irurzun, A. and Barco, A. (2002) in: Molecular biology of picornavirus (Semler, B. L., Wimmer, E. Eds.) pp 337-354, ASM Press, Washington.

36 De Jong, A. S., Wessels, E., Dijkman, H. B., Galama, J. M., Melchers, W. J., Willems, P. H. and van Kuppeveld, F. J. (2003) J.Biol.Chem. 278, 1012-1021.

37 Suhy, D. A., Giddings, T. H., Jr. and Kirkegaard, K. (2000) J.Virol. 74, 8953-8965.

38 Sandoval, I. V., Carrasco, L. (1997) J Virol 71, 4679-4693.

39 Neznanov, N., Kondratova, A., Chumakov, K. M., Angres, B., Zhumabayeva, B., Agol, V. I. and Gudkov, A. V. (2001) J.Virol. 75, 10409-10420.

40 Strauss, J. H., Strauss, E. G. (1994) Microbiol Rev 58, 491-562.

41 Lusa, S., Garoff, H. and Liljestrom, P. (1991) Virology 185, 843-846.

42 Gaedigk-Nitschko, K., Schlesinger, M. J. (1990) Virology 175, 274-281.

43 Liljestrom, P., Garoff, H. (1991) J.Virol. 65, 147-154.

44 Sanz, M. A., Carrasco, L. (2001) J Virol 75, 7778-7784.

45 Sanz, M. A., Perez, L. and Carrasco, L. (1994) J.Biol.Chem. 269, 12106-12110. 46 Schlesinger, M. J., London, S. D. and Ryan, C. (1993) Virology 193, 424-432.

47 Liljestrom, P., Lusa, S., Huylebroeck, D. and Garoff, H. (1991) J Virol 65, 4107-4113.

48 Yao, J. S., Strauss, E. G. and Strauss, J. H. (1996) J Virol 70, 7910-7920.

49 Loewy, A., Smyth, J., von Bonsdorff, C. H., Liljestrom, P. and Schlesinger, M. J. (1995) J Virol 69, 469-475.

50 Gaedigk-Nitschko, K., Ding, M. X., Levy, M. A. and Schlesinger, M. J. (1990) Virology 175, 282-291.

51 Gonzalez, M. E., Carrasco, L. (2001) Virology 279, 201-209.

52 Cohen, E. A., Terwilliger, E. F., Sodroski, J. G. and Haseltine, W. A. (1988) Nature 334, 532-534.

53 Strebel, K., Klimkait, T. and Martin, M. A. (1988) Science 241, 1221-1223.

54 Maldarelli, F., Chen, M. Y., Willey, R. L. and Strebel, K. (1993) J Virol 67, 5056-5061.

55 Schubert, U., Bour, S., Ferrer-Montiel, A. V., Montal, M., Maldarell, F. and Strebel, K. (1996) J Virol 70, 809-819.

56 Chen, M. Y., Maldarelli, F., Karczewski, M. K., Willey, R. L. and Strebel, K. (1993) J Virol 67, 3877-3884.

57 Schubert, U., Schneider, T., Henklein, P., Hoffmann, K., Berthold, E., Hauser, H.,

Pauli, G. and Porstmann, T. (1992) Eur J Biochem 204, 875-883.

58 Jabbar, M. A. (1995) Curr Top Microbiol Immunol 193, 107-20.

59 Kerkau, T., Bacik, I., Bennink, J. R., Yewdell, J. W., Hunig, T., Schimpl, A. and Schubert, U. (1997) J Exp Med 185, 1295-1305.

60 Gonzalez, M. E., Carrasco, L. (1998) Biochemistry 37, 13710-13719.

61 Willbold, D., Hoffmann, S. and Rosch, P. (1997) Eur J Biochem 245, 581-588.

62 Ewart, G. D., Sutherland, T., Gage, P. W. and Cox, G. B. (1996) J Virol 70, 7108-7115.

63 Schubert, U., Ferrer-Montiel, A. V., Oblatt-Montal, M., Henklein, P., Strebel, K. and Montal, M. (1996) FEBS Lett 398, 12-18.

64 Ewart, G. D., Mills, K., Cox, G. B. and Gage, P. W. (2002) Eur.Biophys.J. 31, 26-35.

65 Lamb, R. A., Pinto, L. H. (1997) Virology 229, 1-11.

66 Coady, M. J., Daniel, N. G., Tiganos, E., Allain, B., Friborg, J., Lapointe, J. Y. and Cohen, E. A. (1998) Virology 244, 39-49.

67 Cordes, F. S., Kukol, A., Forrest, L. R., Arkin, I. T., Sansom, M. S. and Fischer, W. B. (2001) Biochim.Biophys.Acta 1512, 291-298.

68 Cordes, F. S., Tustian, A. D., Sansom, M. S., Watts, A. and Fischer, W. B. (2002) Biochemistry 41, 7359-7365.

69 Klimkait, T., Strebel, K., Hoggan, M. D., Martin, M. A. and Orenstein, J. M. (1990) J Virol 64, 621-629.

70 Pinto, L. H., Holsinger, L. J. and Lamb, R. A. (1992) Cell 69, 517-528.

71 Kelly, M., Cook, J. A., Brown-Augsburger P., Heinz, B. A., Smith M. C., Pinto, L. H. (2003) FEBS Lett. 552, 61-67.

72 Mould, J. A., Drury, J. E., Frings, S. M., Kaupp, U. B., Pekosz, A., Lamb, R. A.

and Pinto, L. H. (2000) J.Biol.Chem. 275, 31038-31050.

73 Hay, A. J. (1992) Semin Virol 3, 21-30.

74 Sakaguchi, T., Leser, G. P. and Lamb, R. A. (1996) J.Cell Biol. 133, 733-747.

75 Henkel, J. R., Weisz, O. A. (1998) J.Biol.Chem. 273, 6518-6524.

76 Henkel, J. R., Gibson, G. A., Poland, P. A., Ellis, M. A., Hughey, R. P. and Weisz, O. A. (2000) J Cell Biol. 148, 495-504.

77 Henkel, J. R., Apodaca, G., Altschuler, Y., Hardy, S. and Weisz, O. A. (1998) Mol.Biol.Cell 9, 2477-2490.

78 Hay, A. J., Wolstenholme, A. J., Skehel, J. J. and Smith, M. H. (1985) EMBO J. 4, 3021-3024.

79 Ruigrok, R. W., Hirst, E. M. and Hay, A. J. (1991) J.Gen.Virol. 72, 191-194.

80 Guinea, R., Carrasco, L. (1994) FEBS Lett. 343, 242-246.

81 Guinea, R., Carrasco, L. (1994) FEBS Lett. 349, 327-330.

82 Watanabe, T., Watanabe, S., Ito, H., Kida, H. and Kawaoka, Y. (2001) J.Virol. 75, 5656-5662.

83 Takeda, M., Pekosz, A., Shuck, K., Pinto, L. H. and Lamb, R. A. (2002) J.Virol. 76, 1391-1399.

84 Perez, M., Garcia-Barreno, B., Melero, J. A., Carrasco, L. and Guinea, R. (1997) Virology 235, 342-351.

85 Bodelon, G., Labrada, L., Martinez-Costas, J. and Benavente, J. (2002) J Biol.Chem. 277, 17789-17796.

86 Carrere-Kremer, S., Montpellier-Pala, C., Cocquerel, L., Wychowski, C., Penin, F. and Dubuisson, J. (2002) J.Virol. 76, 3720-3730.

87 Griffin, S. D., Beales, L. P., Clarke, D. S., Worsfold, O., Evans, S. D., Jaeger, J., Harris, M. P. and Rowlands, D. J. (2003) FEBS Lett. 535, 34-38.

88 Harada, T., Tautz, N. and Thiel, H. J. (2000) J.Virol. 74, 9498-9506.

89 Van Etten, J. L., Meints, R. H. (1999) Annu.Rev.Microbiol. 53, 447-494.

90 Plugge, B., Gazzarrini, S., Nelson, M., Cerana, R., Van Etten, J. L., Derst, C., DiFrancesco, D., Moroni, A. and Thiel, G. (2000) Science 287, 1641-1644.

91 Moroni, A., Viscomi, C., Sangiorgio, V., Pagliuca, C., Meckel, T., Horvath, F., Gazzarrini, S., Valbuzzi, P., Van Etten, J. L., DiFrancesco, D. and Thiel, G. (2002) FEBS Lett. 530, 65-69.

92 McWilliam, S. M., Kongsuwan, K., Cowley, J. A., Byrne, K. A. and Walker, P. J. (1997) J.Gen.Virol. 78, 1309-1317.

93 Betakova, T., Wolffe, E. J. and Moss, B. (2000) J.Virol. 74, 4085-4092.

94 Young, R., Wang, I. and Roof, W. D. (2000) Trends Microbiol. 8, 120-128.

95 Alzhanova, D. V., Hagiwara, Y., Peremyslov, V. V. and Dolja, V. V. (2000) Virology 268, 192-200.

96 Bour, S., Perrin, C. and Strebel, K. (1999) J.Biol.Chem. 274, 33800-33806.

97 Ulug, E. T., Garry, R. F. and Bose, H. R., Jr. (1989) Virology 172, 42-50.

98 Barco, A., Carrasco, L. (1995) Embo J 14, 3349-3364.

99 van Kuppeveld, F. J., Melchers, W. J., Kirkegaard, K. and Doedens, J. R. (1997) Virology 227, 111-118.

100 van Kuppeveld, F. J., Hoenderop, J. G., Smeets, R. L., Willems, P. H., Dijkman, H. B., Galama, J. M. and Melchers, W. J. (1997) EMBO J. 16, 3519-3532.

FIGURE LEGENDS

Figure 1. Sequences of selected viroporins. Boxes indicate hydrophobic regions. Basic amino acid residues are shown in bold and some aromatic residue clusters are underlined.

Figure 2. Schematic representation of the pore formed by viroporins.

Table 1. List of several viroporins indicating the number of amino acid residues and some references.

Virus family	Viroporin	AA residues	References
Picornaviridae	Poliovirus 2B	97	[29,30,32,98]
	Coxsackievirus 2B	99	[27,99,100]
	Poliovirus 3A	87	[29,30]
Togaviridae	SFV 6K	60	[45]
	Sindbis virus 6K	55	[17]
	Ross River virus 6K	62	[15]
Retroviridae	HIV-1 Vpu	81	[60,62]
Paramyxoviridae	HRSV SH	64	[84]
Orthomyxoviridae	Influenza A virus M2	97	[13,70,72,80]
Reoviridae	ARV p10	98	[85]
Flaviviridae	HCV p7	63	[86,87]
Phycodnaviridae	PBCV-1 Kcv	94	[90,91]
Rhabdoviridae	BEFV alpha 10p	88	[92]

SFV: Semliki forest virus

HIV-1: Human immunodeficiency virus type 1

HRSV: Human respiratory syncytial virus

ARV: Avian reovirus

HCV: Hepatitis C virus

PBCV-1: *Paramecium bursaria* chlorella virus

BEFV: Bovine ephemeral fever virus

Human Poliovirus 1 protein 2B

GITNYIESLGAAFGSGFTQQIGDKVTELTNMVTSTITEKLLKNLVKIISSLVIIT**R**NYEDTT
TVLATLALLGCDVSPWQWL**KKK**ACDILEIPYAI**K**Q

Human coxsackievirus B3 protein 2B

GV*K*DYVEQLGNAFGSGFTNQICEQVNLLKESLVGQDSILEKSLKALVKIISALVIVV**R**NH
DDLITVTATLALIGCTSSPWRWL**K**Q**K**VSQYYGIPMAE**R**Q

Human Poliovirus 1 protein 3A

GPLQY**K**DL**K**IDI**K**TSPPPECINDLLQAVDSQEV**R**DYCE**KK**GWIVNITSQVQTE**R**NIN**R**A
MTILQAVTTFAAVAGVVYVMY**K**LFAGHQ

Semliki Forest Virus protein 6K

ASVAETMAYLWDQNQALFWLEFAAPVACILIITYCLRNVLCCCKSLSFLVLLSLGATA**R**A

Sindbis virus protein 6K

ETFTETMSYLWSNSQPFFWVQLCIPLAAFIVLMRCCSCCLPFLVVAGAYLA**K**VA

Ross River virus protein 6K

GSASFAETMAYLWDENKTLFWMEFAAPAAALALLACCIKSLICCCKPFSFLVLLSLGAS A**K**A

Human immunodeficiency virus type1 protein Vpu

MQPIQIAIVALVVAIIIAIVVWSIVIIEY**RK**IL**R**Q**RK**ID**R**LID**R**LIE**R**AEDSGNESEGEISALVE
MGVEMGHHAPWDVDDL

Human respiratory syncytial virus protein SH

FWPYFTLIHMITTIISLLIIISIMTAIL

MENTSITIEFSSKN**K**LCEYNVFHN**K**TFELP**R**A**R**VNT

Influenza A virus protein M2

MSLLTEVETPIRNEWGCRCNDSSDPLVVAASIIGILHLILWILD**R**LFF**K**CIY**R**FFEHGL**KR**
GPSTEGVPESM**R**EEY**RK**EQQSAVDADDSHFVSIELE

Avian orthoreovirus protein p10

MLRMPPGSCNGATAVFGNVHCQAAQNTAGGDLQATSSIIAYWPYLAAGGGFLLIVIIFA
LLYCC**K**A**K**V**K**ADAA**R**SVFH**R**ELVALSSG**K**HNAMAPPYDV

Bovine ephemeral fever virus protein alpha 1

ME**K**GLLSNFWNDF**KR**WSED**RK**VEIVIWWSNLES**K**V**R**LGFWIILIILLGILAIRIAI**K**VYQC
V**K**FTNQGV**KK**I**KR**II**KRKR**SI**KK**Y**RK**T

Hepatitis C virus protein p7

ALENLVILNAASLAGTHGLVSFLVFFCFAWYL**K**G**R**WVPGAVYALYGMWPLLLLLLALP
Q**R**AYA

Paramecium bursaria chlorella virus protein Kcv

PFMIHLFILAMFVMIYKFFPGG FENN
KTTGA**K**LCTIAHIVTVFFIVLTL
MLVFS**K**FLT**R**TEFSVANPD**KK**ASWIDCI
YFGVTTHSTVGFGDILP