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THE ION CHANNELS CODED BY VIRUSES*

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Pathogenicity and virulence are multifactorial traits, depending on interaction of viruses with susceptible cells and organisms. The ion channels coded by viruses, *viroporins*, represent only one factor taking part in the cascade of interactions between virus and cell, leading to the entry of virus, replication and to profound changes in membrane permeability.

The M2 protein from influenza A virus forms proton-selective, pH-regulated channel involved in regulating vesicular pH, a function important for the correct maturation of HA glycoprotein. The NB glycoprotein of influenza B viruses is an integral membrane protein with an ion channel activity. The CM2 protein of influenza C virus is an integral membrane glycoprotein structurally analogous to influenza A virus M2 and influenza B virus NB proteins. The picornavirus 3A protein is involved in cell lysis and shows homology with other lytic proteins. Vpu is an oligomeric integral membrane protein encoded by HIV-1, which forms ion channels. The togavirus 6K protein shows structural similarities with other viroporins.

Keywords: viroporins, influenza virus, ion channels, M2 protein, NB protein, CM2 protein, picornavirus, togavirus, HIV proteins

Introduction

Ion channels are responsible for the rapid and efficient conduction of ions across phospholipid bilayers of cell membranes. Animal viruses permeabilize cell membranes at the early stage during the infection, when the virus penetrates into the cell and during the expression of the virus genome. Early membrane permeability is induced by isolated virus particles, whereas late membrane permeability is produced by newly synthesized virus protein(s) that possess activities resem-

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bling ionophores. The virus products involved in cytopathic effect and virulence have the plasma membrane as the primary target of their toxic effect. The animal viruses encode proteins that form small pores in the lipid bilayer acting as ionophores and represent the new family of virus proteins that enhance membrane permeability: *the viroporins* [1–3].

General properties of viroporins

Viroporin structure:

– short proteins containing around 50–120 amino acid residues
– they contain a higher than average content of leucine plus isoleucine residues and a lower content of glycine
– all of viroporins possess a hydrophobic stretch of about 20 amino acids
– they are integral membrane proteins
– viroporins tend to form oligomers, most frequently tetramers
– viroporins usually contain basic amino acids, participating in membrane permeabilization by destabilizing the lipid bilayer [2, 4].

Viroporin function:

– the main function of viroporins is to help in the release of progeny virus from infected cells
– viruses exit the cell because the membrane is being lysed by specific membrane-active proteins encoded by viral genome
– animal viruses contain in they genomes one or more genes specifically devoted to lysing the plasma membrane
– budding from the plasma membrane, which is modified by a specific viral product that produces pores
– viroporin action at the molecular level may be directed to deenergizing the membrane and disrupting its physical integrity
– formation of pores leads to the dissipation of the membrane potential and the ionic gradients that would be gradually destroyed as infection progresses
– viroporins present in intracellular compartments, including the proton gradient, would disappear
– viroporin activity may also have consequences for cellular metabolism and morphology, as occurs with membrane-active toxins that affect an array of cellular functions by simply modifying membrane function

– monovalent ions in virus-infected cells are responsible for the virus-induced shut-off of host protein translation
– formation of pores is plastic
– pores formed by lytic proteins do not have a determined size
– viroporins are involved in the intracellular regulation of pH and are responsible for conformational changes of viral glycoproteins
– pores are not selective with regard to the molecules that can pass through them [1, 2, 5].

Early and late membrane permeabilization: the result of activity of pathogenicity and virulence factors

A proton motive model predicts that there are viral proteins that open pores in membranes and, together with receptors, are able to use energy to translocate substrates in a non-favorable thermodynamic direction. In contrast to an un-coupler, which simply dissipates the energy stored in ionic gradients, this "virus-transducing complex" is able to couple the energy to genome translocation. The pore size would permit the passage of protons and other ions, but it prevents the diffusion of macromolecules. Virions modify membrane potential during entry, probably because of the capacity of virion proteins to form ion channels [2].

One of the most salient characteristics of the late membrane modifications is that they require viral gene expression, suggesting that one or several virus genes products are responsible for these changes.

Each cytolytic animal virus modifies the permeability of the plasma membrane with different kinetics, most probably reflecting not only the different timing of virus gene expression but also the intrinsic activity of each particular viral protein involved in cell lysis.

The most common change that occurs in the plasma membrane during infection of a permissive cell by a cytolytic virus is enhanced permeability to monovalent cations. This effect is obviously accompanied by a drastic drop in membrane potential. Sodium ions that are pumped outside the cell by Na^+–K^+–ATPase readily enter into infected cells, whereas potassium ions that accumulate inside the cell leak out. In the case of picorna- or togaviruses these modifications in ion distribution are initially observed 2–3 hrs after infection. On the other hand, modifications in ionic concentrations are inhibitory for the translation of most cellular mRNAs, although the synthesis of a few cellular proteins, such as the heat-shock proteins, is resistant to such modifications.

The concentrations of protons and calcium ions change at about the same time when monovalent ion concentrations are affected [1, 2].

The ion channels coded by influenza viruses

The M2 from influenza virus is an essential component of the viral envelope and forms a highly selective, pH-regulated proton channel. The influenza virus enters cells through internalization into the endocytic pathway, with virus uncoating taking place in endosomal compartments. The M2 ion channel activity permits protons to enter the virion interior, and this acidification weakens the interaction of the matrix protein (M1) with the ribonucleoprotein core. The M2 is a small (97 residues) protein and contains one hydrophobic stretch of 18 residues to form a transmembrane helix [4, 6–8]. Highly pathogenic and virulent avian strains are cleaved by a family of more widespread intracellular proteases resulting in systemic infection. This difference in pathogenicity and virulence correlates with structural differences at the HA0 cleavage site. The cleavage results in structural rearrangements in which the nonpolar amino acids near the new amino-terminus bury ionozable residues in the cavity that are implicated in the low-pH-induced conformation change [9]. Amantadine treatment of cells infected with H7 strains

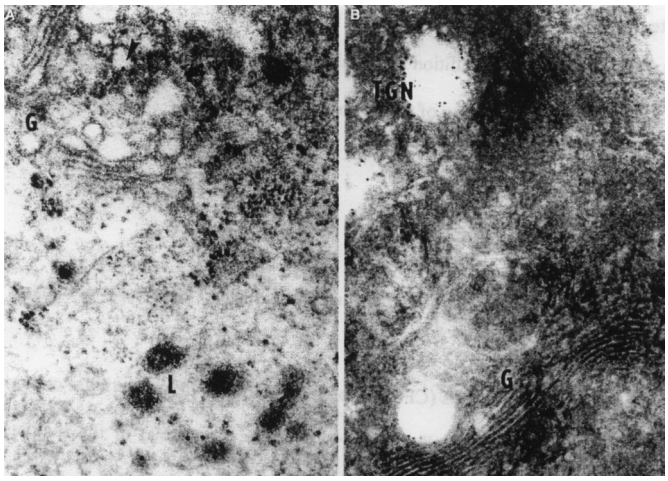

Figure 1. Localization of acidic compartments in uninfected (A) and influenza virus A, strain Rostock-infected (B) MDCK cells. (A) Ultrathin epoxy-resin section of MDCK treated with DAMP and labeled with anti-DNP antibodies, RAM, and GAR 10. Gold particles are localized on primary lysozomes (L) and on trans-Golgi vesicles (arrow); (B) Ultrathin cryosection of Rostock-infected MDCK cells labeled as in (A). Gold particles are associated predominantly with vesicles of the trans-Golgi region (TGN). Bar = 100 nm

of influenza A viruses causes an M2 protein-mediated conversion of haemagglutinin from its native to its low pH conformation (Figures 1 and 2). The structural alteration and hence drug action occur shortly after haemagglutinin exits from the Golgi complex during its passage through the trans-Golgi region. The alteration in haemagglutinin is the direct consequence of exposure to an adverse low pH and provide support that the M2 proteins are involved in regulating vesicular pH, a function important for the correct maturation of haemagglutinin glycoprotein [5, 7, 10–17].

The NB protein is an integral component of the membrane of influenza B virus. The similarities between NB of influenza B and M2 of influenza A viruses in structural features, their presence in the virion and possession of an ion channel activity suggest, by analogy with the M2 protein, that NB may also have a role in virus entry, pathogenicity and virulence. NB protein contains a single hydrophobic sequence of 19 amino acids. NB forms ion channels when inserted in artificial lipid bilayers. The channel activity can be blocked by amantadine at concentrations that inhibit replication of the influenza B virus [18–20].

The CM2 protein of influenza C virus is an oligomeric integral membrane glycoprotein structurally analogous to influenza A virus M2 and influenza B virus NB proteins. CM2 is encoded within the CM2 ORF on influenza C virus RNA segment 6. There is little information about the transport of the influenza C virus HEF glycoprotein through the acidic lumen of the trans-Golgi network (TGN). The available data indicate that CM2 has a cleavable signal peptide at the N-terminus of the protein. Cell surface biotinylation and indirect immunofluorescence showed the protein to be expressed at the cell surface [21].

Picornaviruses

Picornavirus 3A protein is the poliovirus protein involved in cell lysis. Polypeptid 3A, and its precursor 3AB, are known to interact with cellular membranes in infected human cells. Computer analyses of these proteins indicated that the hydrophobicity of 3A extended over a region of 20–22 amino acids that formed an amphipathic helix. Moreover, 2A is a basic protein that shows homology with other lytic proteins [2].

Coxackievirus protein 2B modifies endoplasmic reticulum membrane and plasma membrane permeability and facilitates virus release. Van Kuppeveld et al. [22] propose that 2B by forming membrane-embedded pores gradually enhances membrane permeability, thereby disrupting the intracellular Ca^{2+} homeostasis and ultimately causing the membrane lesions that allow release of virus progeny.

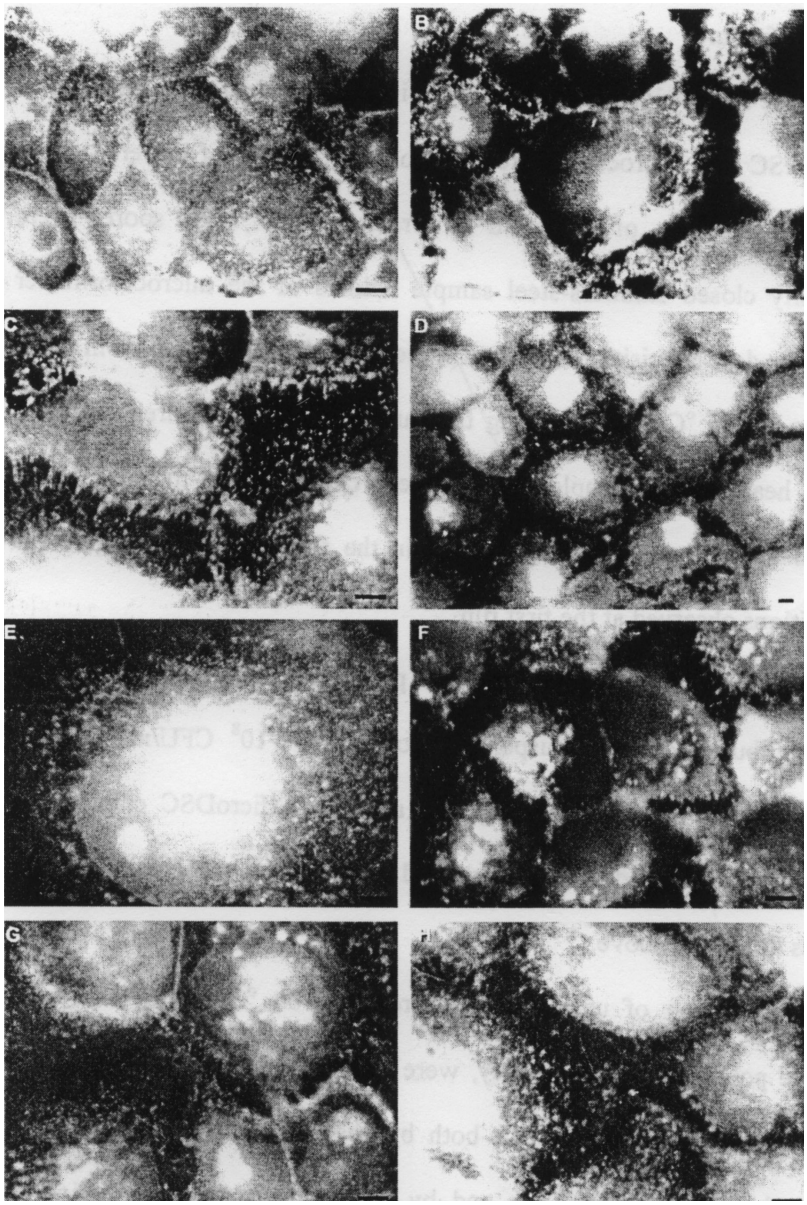

Figure 2. Immunofluorescent localization of native and low pH HA in amantadine-treated infected cells. MDCK cells, 5 ½ hrs p.i. were fixed and incubated with antibody HC2 (A, B: recognizes all conformations of HA), the native conformation HA-specific antibody, HC58 (C, D, G), or the low pH HA-specific antibody H9 (E, F, H) followed by RAM-FITC. A–F: cells infected with Rostock; G, H: cells infected with amantadine resistant strain of Rostock; A, C, E: no amantadine; B, D, F, G, H: amantadine (5 μM) added 1 hr p.i. Bar = 0.02 mm

Ion channels formed by HIV-1 Vpu

HIV proteins modify membrane permeability. Alterations of membrane permeability by HIV infection are observed early during infection. The late alterations include nonspecific modifications of the transport of low-molecular-weight compounds and monovalent and divalent cations. Portions of the HIV gp41 increase membrane permeability. The transmembrane domain of this protein shows similarities to other viroporins. Vpu is a typical viroporin molecule. It is an integral membrane protein containing 81 amino acids and is phosphorylated on a serine residue. It contains a hydrophobic stretch of 28 amino acids at the amino terminus embedded in the membrane, with the hydrophilic carboxy terminus oriented toward the cytoplasm. Vpu is produced late in replication and it has not been found in virions. Vpu is localized in a perinuclear region, most likely in the Golgi complex. Virions from Vpu-negative provirus accumulate at the cell surface and in endosomes. Similarities of Vpu with other amphipathic viral proteins, including influenza M2, have been described. The N-terminal transmembrane domain is largely responsible for channel formation and for promotion of virus release [2, 23, 24].

Ion channels formed by togaviruses

Togavirus 6K protein. Togaviruses induce drastic changes in membrane permeability during infection. The 6K protein shows structural similarities with other viroporins. The togavirus 6K protein is a hydrophobic membrane protein that increases membrane permeability and induces cell lysis [2].

Other putative viroporins

Analyses of hydrophobicity plots and the actual hydrophobic sequences indicate that coronavirus IBV-D3, IA RSV, E3-11,6 K-adeno 2, HU-papilloma E5, BPV-1 proteins are membrane active proteins and can have a function of viroporins [2]. Rotavirus nonstructural glycoprotein NSP4 alters membrane permeability in mammalian cells. Expression of NSP4 in *Spodoptera frugiperda* cells by using a recombinant baculovirus shows that the polypeptide induced a rise in intracellular free calcium concentration. NSP4 might share features with several other viral proteins that can modify membrane permeability and thus facilitate the release of progeny virions into the medium [25, 26].

Conclusions

The recent rapid progress in understanding the composition of viroporins, their spatial organisation and functions has focused much attention on the interaction of viruses with susceptible cells, converting them into one of the central fields of interest in modern virology and cell biology.

The term "viroporins" is commonly used to define several types of viral coded proteins with ion channel activity. The modifications in membrane permeability produced during virus infection are clearly defined. Early membrane permeabilization is caused by viral particles during the process of virus entry and uncoating. Insights into the molecular mechanism of this process suggest that animal viruses require a proton-motive force to enter cells and to permeabilize them. In this respect, viroporins represent one very important chain in multifactorial traits of pathogenicity and virulence.

Late membrane leakiness requires virus gene expression and is manifested as general increase in permeability to ions and low-molecular-weight compounds, suggesting that physical pores in the membrane are generated during virus infection. Those pores would arise by oligomerization of particular viral proteins designed to lyse the infected cells.

Novel results indicate that new viroporins can be described in other than known viral proteins. In view of this pattern, new mechanisms of control and regulation of chemical and molecular biological reactions at all stages of virus morphogenesis become conceivable. Since viral replication has indicated a clear involvement of the viroporins in virus metabolism, further biochemical, molecular biological and morphological studies are needed to establish more precise correlations between the numerous ultrastructural changes described in virus-infected cells. The way is now open to analyze the effects that viroporins have different cellular functions, such as inhibition of host macromolecular synthesis and the cytopathic effect. In addition, the avenues are open for further investigation.

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