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Viruses infecting marine picoplancton encode functional potassium ion channels

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ABSTRACT

Phycodnaviruses are dsDNA viruses, which infect algae. Their large genomes encode many gene products, like small K^+ channels, with homologs in prokaryotes and eukaryotes. Screening for K^+ channels revealed their abundance in viruses from fresh-water habitats. Recent sequencing of viruses from marine algae or from salt water in Antarctica revealed sequences with the predicted characteristics of K^+ channels but with some unexpected features. Two genes encode either 78 or 79 amino acid proteins, which are the smallest known K^+ channels. Also of interest is an unusual sequence in the canonical α-helixes in K^+ channels. Structural prediction algorithms indicate that the new channels have the conserved α-helix folds but the algorithms failed to identify the expected transmembrane domains flanking the K^+ channel pores. In spite of these unexpected properties electophysiological studies confirmed that the new proteins are functional K^+ channels.

Introduction

Several viruses have genes that encode proteins with ion channel activity (e.g., Fischer and Sansom, 2002; Wang et al., 2010; Nieva et al., 2012). Depending on the virus, these channels are either involved in viral entry, viral replication or viral exit from the host (Hsu et al., 2004; Thiel et al., 2009; Nieva et al., 2012). Bioinformatic analyses of these viral encoded channels have not revealed any obvious sequence similarities to channel proteins from cellular organisms (Fischer and Sansom, 2002). One exception is viruses in the family phycodnaviridae, which code for proteins with the structural and functional hallmarks of K^+ channels (Plugge et al., 2000; Thiel et al., 2011). The prototype K^+ channel Kcv is coded by chlorovirus PBCV-1 (Kcv_{PBCV-1}) (Plugge et al., 2000) and like its prokaryotic and eukaryotic homologs, it functions as a tetramer (Shim et al., 2007; Pagliuca et al., 2007). Each Kcv monomer has two transmembrane domains (TMDs), which are linked by a pore helix (Tayefeh et al., 2009). The pore helix contains a motif of 8 amino acids, which is the signature sequence for all K^+ channels (Heginbotham et al., 1994). Assembly of four Kcv monomers creates a central pore with a selectivity filter that allows passage of K^+ across the membrane (Tayefeh et al., 2009). The major difference between Kcv_{PBCV-1} and K^+ channels from other organisms is the small size of the monomers, which are only 94 amino acids (Plugge et al., 2000); that is, Kcv_{PBCV-1} basically consists of the pore module present in all K^+ channels (Thiel et al., 2011). However, in spite of its small size the Kcv_{PBCV-1} channel has all the functional hallmarks of more complex K^+ channels when expressed in heterologous systems, including selectivity for K^+ and sensitivity to many of the known K^+ channel blockers (Thiel et al., 2011).

After discovering Kcv_{PBCV-1}, we realized that K^+ channel encoding genes are common in members of the Phycodnaviridae family. K^+ channel sequences have been detected in more than 80 phycodnaviruses (Kang et al., 2004; Gazzarrini et al., 2006, 2009; Hamacher et al., 2012; Thiel and Van Etten, unpublished data). From an evolutionary point of view it is interesting that K^+ channel coding sequences are found in members, representing four genera in the Phycodnaviridae; these viruses infect different algal hosts. Three of these viruses replicate in species of unicellular green algae from fresh water habitats, Chlorella variabilis, Chlorella heliozoae, and Micractinium conductrix (Fitzgerald et al., 2007a, 2007b, 2007; Jeanniard et al., 2013); collectively these viruses are called chloroviruses. A fourth virus, EsV-1, also encodes a K^+ channel protein named Kesv. EsV-1 infects the marine filamentous brown alga Ectocarpus siliculosus; EsV-1 is distantly

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related to the viruses that infect fresh water green algae (Van Etten et al., 2002). Several studies have established that the Kcv channels are located in the internal membrane of the chloroviruses (Romani et al., 2013; Frohns et al., 2006) and that they serve an important role in the early steps of infection and DNA ejection into the host (Neupärtl et al., 2008; Greiner et al., 2009). The biological role of the K^+ channel in the marine EsV-1 is unknown but presumably it is different from that in the chloroviruses. The reason for this assumption is that chloroviruses have a lytic life cycle while EsV-1 is lysogenic (Delaroque et al., 1999; Van Etten et al., 2002). Also the energetic barrier for ejecting viral DNA into the host, which is lowered by Kcv activity in the fresh water algae (Neupärtl et al., 2008; Thiel et al., 2009), is not relevant in the marine habitat because virus EsV-1 infects the sporophytes of the host cells, which lack a cell wall and hence have no turgor pressure (Delaroque et al., 1999).

Although, the K^+ channels from phycodnaviruses are similar, they do exhibit significant structural and functional diversity. An obvious structural difference is their monomer size, which ranges from 124 amino acids in the Kesv channel from virus EsV-1 (Balss et al., 2008) to 82 amino acids in viruses, which infect Chlorella heliozoae (Gazzarrini et al., 2009). These size differences are mostly due to the presence or absence of cytoplasmic domains and an extracellular turret domain in the channels (Thiel et al., 2009). Diversity also exists in the functional properties of the channels when they are expressed in heterologous systems. For example, Kcv_{PBCV-1} has a lower open probability than the corresponding channel from chlorovirus ATCV-1, Kcv_{ATCV-1}. Also, Kcv_{PBCV-1} conducts Rb^+ better than K^+ whereas the situation is reversed in Kcv_{ATCV-1} (Gazzarrini et al., 2009). In addition to their functional differences the K^+ channels are sorted differently. The chlorovirus encoded Kcv channels are sorted into the secretory pathway and finally targeted to the plasma membrane in either HEK293 cells or in yeast, the Kesv channel from EsV-1 is targeted to the mitochondria (Balss et al., 2008).

Another interesting question is the origin and the evolution of the viral K^+ channel proteins. The fact that all K^+ channels from cellular organisms contain a pore, which resembles the viral K^+ channels, is consistent with the traditional assumption that viruses are 'pick pockets' (Moreira and Lopez-Garcia, 2009) and acquire their genes from their host via molecular piracy. However, this traditional view on the evolution of viral K^+ channels has been challenged recently. Comparative analysis of the Kcv channels from different chloroviruses and from virus EsV-1 with those coded by the two host cells found no evidence of co-evolution between the viruses and their hosts (Hamacher et al., 2012). Instead, a phylogenetic analysis indicated that the viral channels form, in spite of their structural and host diversities, a defined clade; i.e., the viral channels are clearly separated from their host K^+ channels and from K^+ channels from other cellular organisms (Thiel et al., 2013). This analysis clearly argues against the hypothesis that viruses have acquired their K^+ channels from their current hosts. This conclusion is further supported by a bioinformatics analysis of 41 chloroviruses with one of their hosts. The results of this study did not find any evidence to indicate a major transfer of genes from the host to the chloroviruses. For a few genes the results even indicated a flow of genes in the opposite direction, i.e., from virus to host (Jeanniard et al., 2013).

In the context of the question about the origin of viral K^+ channels, recent sequencing projects of viruses infecting marine unicellular algae (Moreau et al., 2010; Derelle et al., 2006, 2008) and metagenomic sequencing of an organic lake in Antarctica (Zhou et al., 2013; Yau et al., 2011) revealed open reading frames that were annotated as K^+ channels. Furthermore, in the context of the minimal size required for a functional K^+ channel, two of the newly detected putative K^+ channels have a monomer size of 78 and 79 amino acids, which is even smaller than the 82 amino acid Kcv_{ATCV-1} channel (Gazzarrini et al., 2009). In this manuscript we report a detailed structural and functional examination of three of the new putative K^+ channel proteins, as well as their phylogenetic relationships. The results revealed considerable variability among the viral K^+ channels; a phylogenetic analysis indicated that the K^+ channels from the fresh water viruses clearly separated from those from the marine/salt water habitats. These results support the notion of a long evolutionary history for the viral K^+ channels.

Results and discussion

New virus encoded K^+ channels

Fig. 1A shows an alignment of eight newly detected putative K^+ channel sequences from viruses infecting algae. Seven of the viruses with K^+ channel like sequences infect small unicellular algae, which are the main components of the so-called picoplankton community. Their hosts, *Micromonas*, *Bathycoccus* and *Osterococcus* species, belong to the class *Prasinophyceae* within the *Chlorophyta*; these algae are important ecologically because they are often the dominant photosynthetic species in marine habitats. Four of the viruses (MpV12T, MpVSP1, MpV1, MpvPL1) infect *Micromonas pusilla*. Two viruses (BpV1, BpV2) infect *Bathycoccus* species; the sequence of the putative K^+ channel protein from these two viruses is identical. Two additional viruses (OlV4, ORT) infect *Osterococcus* species. The name of the gene products in Fig. 1A is composed of K for K^+-channel, and the virus, which encodes the sequence, e.g., mpv is for *M. pusilla* virus; the index specifies the virus isolate. Thus $Kmpv_{12T}$ is the K^+ channel from *M. pusilla* virus isolate 12 T. Finally, a K^+ channel like sequence was detected in a metagenomic sequencing project of viral genomes in an organic lake in Antarctica (Yau et al., 2011). In this case, neither the host nor the virus encoding the K^+ channel from the organic lake phycodnavirus 2, $Kolpv_2$, is known. Information on the gene accession numbers, on the source of the genes and on the protein nomenclature are summarized in Table 1.

Table 1
Gene accession numbers, viral source of genes, nomenclature of putative K^+ channels, protein accession numbers and protein sizes.

Gene accession number of virus genom	From virus	Name of putative K^+ channel	Protein accession number	Number of amino acids
HM004429	*Micromonas* sp. *RCC1109 virus MpV1*	$Kmpv_1$	YP_004062056	79
HQ632826	*Micromonas pusilla virus 12T*	$Kmpv_{12T}$	YP_007676152	78
JF974320	*Micromonas pusilla virus SP1*	$Kmpv_{SP1}$	AET84893	86
HQ633072	*Micromonas pusilla virus PL1*	$Kmpv_{PL1}$	AET43568	85
HM004432	*Bathycoccus* sp. *RCC1105 virus BpV1*	$Kbpv_1$	YP_004061440	83
JF974316	*Ostreococcus lucimarinus virus OlV4*	$Kolv_4$	AET84496	102
JN225873	*Ostreococcus tauri virus RT-2011*	$Kotv_{RT}$	AFC34969	104
HQ704803	*Organic Lake phycodnavirus 2*	$Kolpv_2$	ADX06223	105