

## Introduction

Viral channels or viroporins are a group of small (60 – 120 amino acids) viral encoded proteins, that enable the passage of ions and small molecules through the membrane of host cells.

Viral channels are not essential for the replication of the virus. However, they can promote virus growth by changing the permeability of the membrane and thereby the milieu of the cell or by providing a pathway for the release of viral particles.

Prominent members of virus encoded porins that build pores or ion channels are:

- **Avian Influenza A Virus**

The M2 protein is a small transmembrane protein of 97 amino acids. The functional channel is a homotetramer of four identical subunits. Patch clamp experiments showed that it is a proton selective ion channel. It can be blocked by the anti-influenza drugs amantadine and rimantadine.

- **Alphavirus**

Alphaviruses are of particular interest because members of this genus, e.g. the Ross River Virus (RRV) and the Barmah Forest Virus (BFV), are the origin of epidemic polyarthritits in Australia and are transmitted by the mosquito. The 6K proteins of alphaviruses are small hydrophobic proteins (58 – 61 amino acids) that associate with membranes. For the RRV and the BFV it was shown that the 6K proteins form cation selective ion channels. They show in planar lipid bilayers a very heterogenic single channel conductance that varies between 40 - 800 pS, probably due to a wide range of different oligomerization states.

- **Poliovirus**

2B, a pore forming protein, is permeable for small molecules < 1000 Da. Probably four molecules form a homotetramer.

- **HIV-1**

Vpu is a small protein of 81 amino acids. It forms a pentamer with ion channel activity and is essential for particle release, virus load and degradation of the surface marker CD4.

- **Chlorellavirus**

Kcv is a potassium selective ion channel from *Paramecium bursaria* Chlorella virus (PBCV-1). The open reading frame of the gene encodes a small protein of 94 amino acids. Kcv is the first viral ion channel characterized in detail.

Research on viral channels and porins is just at the beginning and in the near future, this group of proteins and the knowledge about their function, regulation and pharmaceutical influence will grow. As with other ion permeable proteins, unequivocal electrophysiological characterization and pharmacological investigation of viroporins will be a main focus of research and the **Ionovation Compact** the tool of choice.

## Example: Kcv, a small viral potassium channel

*Paramecium bursaria* chlorella virus 1 (PBCV-1) is a double-stranded DNA containing virus that infects certain eukaryotic chlorella-like green algae.

Sequence analysis of the genome revealed an open reading frame encoding a small protein (94 amino acids) named Kcv with similarities to the eukaryotic family of the two transmembrane domain potassium channels (Kang et al., Proc Natl Acad Sci USA 2004: 101, 5218-24).

Due to its minimalistic features Kcv is an interesting and suitable model system.

Kcv is the first viral encoded  $K^+$  channel discovered and the smallest known functional  $K^+$  channel.

It is a minimal channel, mainly consisting only of the structure absolutely needed for function, the pore, surrounded by a  $\alpha$ -helical transmembrane region on each side.

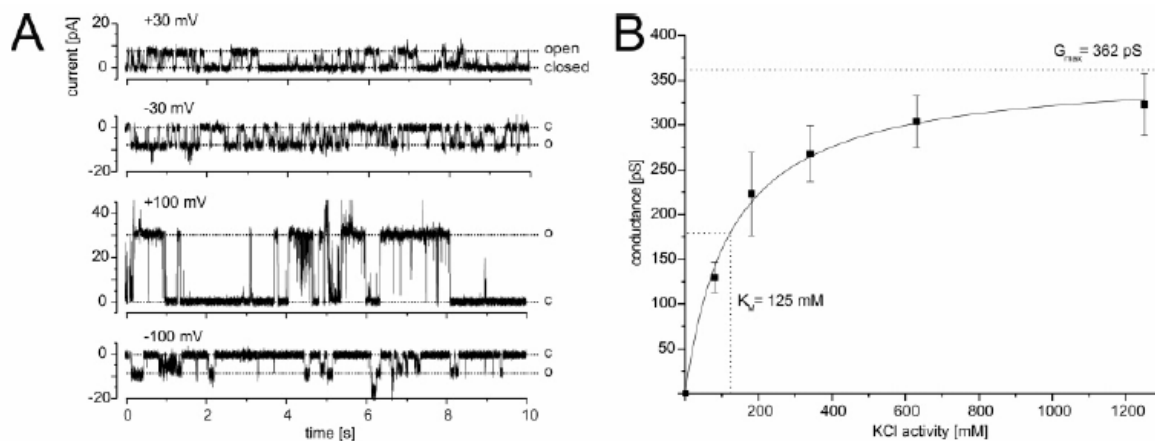
Despite its small size, Kcv has been shown to be a functional channel in heterologous expression systems as well as in planar lipid bilayers.

Kcv plays an important role during viral infection and most likely is essential for the replication of PBCV-1.

Incorporation of Kcv into the plasmamembrane of the host cell leads to  $K^+$  efflux and therefore a depolarization of the membrane, presumably followed by cell wall degradation and the ejection of viral DNA.

Electrophysiological characterization in planar bilayers revealed a saturating single channel conductance of 360 pS, high  $K^+$  selectivity, and block by  $Ba^{2+}$ ,  $Cs^+$  and  $Na^+$  (Pagliuca et al., Biochemistry 2007; 46(4):1079-90). A block by the anti-influenza drug amantadine was also described.

The channel protein is available in huge amounts and therefore makes it an ideal candidate for detailed structure-function studies.



### Single-channel conductance properties of the reconstituted Kcv protein.

A) Single-channel currents recorded under symmetrical conditions, cis/trans 500 mM KCl with 10 mM MOPS/Tris (pH 7.0), at +30 and -100 mV. Dotted lines denote corresponding current levels of the closed and fully open conformations of Kcv. A pronounced rectifying behavior becomes obvious when current levels at 100 mV and -100 mV are compared.

B) Conductance of the Kcv channel at different KCl activities, under symmetrical conditions, cis/trans 10 mM MOPS/Tris (pH 7.0), with the indicated KCl activity.