Structure/Function Analysis of the Viral Potassium Channel Kcv:
The importance of the two transmembrane domains

Manuela Gebhardt1, Sascha Tayefeh1, Franziska Hoffgaard2, Kay Hamacher2, Dirk Baumeister1, Brigitte Hertel1, Timo Greiner1, James L. Van Etten3, Stefan M. Kast4, Anna Moroni5, Gerhard Thiel1,6

1Inst. Botany TU-Darmstadt, Germany, 2Computational Biology Group TU-Darmstadt, 3Department of Plant Pathology and Nebraska Center for Virology, University of Nebraska, Lincoln, NE 68583-0722, USA, 4Physikalische Chemie III, Technische Universität Dortmund, Germany, 5Department of Biology and CNR IBF-Mi, Università degli Studi di Milano, Italy, 6Loewe cluster Soft-Control, Technische Universität Darmstadt, Germany

Address correspondence to: gebhardt@bio.tu-darmstadt.de

The potassium channel KcvPBCV-1 from the virus PBCV-1 is with only 94 amino acids very small. Still it exhibits many structural and functional hallmarks of complex potassium channels. Here we analysed the importance of the two transmembrane domains (TMD1 and TMD2) for channel function. A combination of Ala scanning, yeast complementation assays, electrophysiological recordings and computational studies provide interesting insights into the functional architecture of the channel.

TMD1 of KcvPBCV-1 contains a Lys at the membrane/polar interface. This is typical for many transmembrane domains and it is believed that the long side chains of the Lys can “snorkel”; they can keep their aliphatic part in the hydrophobic lipid bilayer and position the charged terminal group in the polar interface. An Ala substitution and further mutations of Lys29 in KcvPBCV-1 shows that the cationic amino acid, which is highly conserved in Kcv type channels, can indeed snorkel. This activity however is not essential in channels in which the outer transmembrane domain is anchored in the membrane. Only in channels, which lack a significant N-terminal domain, the snorkeling becomes essential for a proper positioning of the TMD in the membrane and hence for function. These channels are as a consequence more sensitive to a neutralization of the respective cationic amino acid.

The importance of a proper positioning of the TMDs in the bilayer becomes also evident from the full Ala scanning of the two TMDs in combination with MD simulations. It appears that the upper halves of both TMDs, which are facing towards
the external medium, are rather rigid while the inner parts are more flexible. The rigidity of the outer TMD is conferred by a number of essential aromatic amino acids, which face the membrane and probably anchor this domain in the bilayer. The inner TMD is intimately connected with the rigid part of the outer TMD via π-π-interactions between a pair of aromatic amino acids. This structural principle is conserved within the viral K⁺ channels but also present in Kir2.2 implying a general importance of this architecture for K⁺ channel function.