Analysis of the Ion Channel Gating Mechanism in Solution by Nuclear Magnetic Resonance (NMR) Spectroscopy

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Ion channels, which can be activated by binding of cyclic nucleotides, play a crucial role in the regulation of the excitation of cardiac muscle cells, and they are of importance in signal transduction of olfactory and visual neurons. All of these channels belong to the class of voltage-gated cation channels, which can be sub-classified in cyclic nucleotide-gated ion channels (CNG) and hyperpolarization-activated and cyclic nucleotide-gated ion channels (HCN) (Schünke and Stoldt 2013).

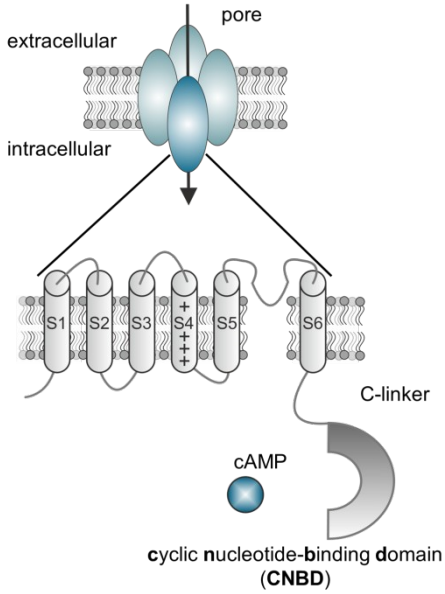

Figure 1: Subunit topology of cyclic nucleotide-activated ion channels. HCN and CNG channels consist of four subunits. Each subunit contains a six trans-membrane segment (S1-S6), an intra-cellular cyclic nucleotide-binding domain and a connecting C-linker.

For CNG channels the binding of cyclic nucleotides is important for the opening of the membrane pore whereas they are only weakly dependent on membrane depolarization. Cyclic nucleotides, bound to the cyclic nucleotide-binding domain of HCN channels, solely modulate the opening behaviour. In this case, the voltage, necessary for the pore opening, is shifted to more positive values (Schünke and Stoldt 2013).

Upon binding of the cyclic nucleotide to the CNBD, the signal is transferred to the membrane domain, in order to open the pore or to change the opening behaviour. To analyse the underlying gating mechanism three-dimensional structures at atomic resolution are indispensable.

Up to now, several crystal structures of eukaryotic HCN CNBDs are available. However, comparison of cyclic nucleotide free and bound structures did not reveal significant differences (Zagotta et al. 2003 and Taraska et al. 2009). In contrast, structures of the CNBD from the prokaryotic organism Mesorhizobium loti, solved by liquid state NMR spectroscopy, did show substantial rearrangements upon binding of a cyclic nucleotide (Schünke et al. 2009 and Schünke et al. 2011).

Here, we show the first structural analysis of an eukaryotic CNBD using liquid state NMR spectroscopy. We could demonstrate that binding of cyclic nucleotides to the CNBD results in significant conformational changes yielding new insights into the ion channel gating mechanism.

References:

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