Calcium-activated chloride channels (CaCC) play an important role in cellular physiology ranging from fluid secretion in epithelia to the regulation of excitability in muscle and neurons. Channel activation by Ca2+ was shown to be voltage-dependent with an increase of the EC50 at negative potentials. In 2008, CaCC activity was shown to be conferred by one of the ten members of TMEM16 protein family. It was soon established that only two of the members, TMEM16A and TMEM16B, function as CaCCs while some TMEM16 proteins have been characterized as Ca2+-dependent phospholipid scramblase. The ability of the TMEM16 family protein to carry out these two very different physiological functions remained puzzling due the lack of structural information of this family. The aim of the thesis was to determine the first high-resolution structure of a TMEM16 family member and to apply the structural information to understand how TMEM16A functions as a Ca2+-activated chloride channel. To increase the likelihood of obtaining a crystal structure, a broad homologue expression screen was performed in S. cerevisiae. A fungal homologue from Nectria haematococca, nhTMEM16, was identified as a suitable candidate and its crystal structure was subsequently determined in our group. The crystal structure of nhTMEM16 revealed the novel dimeric architecture of TMEM16 protein family and the presence of Ca2+-binding sites within the transmembrane region. The relevance of the Ca2+ binding site in TMEM16A was studied by patch clamp electrophysiology and was utilized in further study to determine the functional independence of the two pores in TMEM16A. The nhTMEM16 structure combined with the structure-function studies on TMEM16A described in this thesis, provide the first mechanistic insight into gating and ion conduction of CaCCs. This work serves as basis for further investigation of the dual functionality found within the TMEM16 family.

Date & time: Tuesday, December 20, 2016 at 04:00 pm
Location: Lecture Hall Y44-H-11, UZH Irchel
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