TMEM9 SUBUNITS AS NEW MOLECULAR REGULATORS OF ENDOSOMAL CHLORIDE/PROTON EXCHANGERS

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Endosomes require tight control of luminal ions, prominently including Cl^- and H^+, for proper trafficking and function. Important regulators are the five distinct CLC chloride/proton exchangers which are differentially expressed in the endolysosomal system. Each of these exchangers is essential for cellular health, as their dysfunction results in severe disease. Here, we have identified TMEM9s (TMEM9 and TMEM9B) as essential β-subunits for the endosomal ClC- 3, ClC-4, and ClC-5 transporters. TMEM9s are required not only for the stability and trafficking of their associated CLCs but also for modulating their ion transport activity. Mice lacking both TMEM9s exhibit dramatically reduced CLC-3 to -5 protein levels and die perinatally, highlighting the physiological importance of the beta-subunits. TMEM9s proteins strongly inhibit CLC ion transport and thereby prevent excessive Cl^- accumulation and endosomal swelling. The inhibitory effect depends on a conserved C-terminal domain in TMEM9s that interacts with multiple sites on the CLCs. Human disease-causing mutants in ClC-3 and ClC-4 which interfere with this binding cause toxic increase of ion transport. Our Cryo-EM analysis of ClC-3/TMEM9 complexes revealed that TMEM9 C-terminus inhibits ClC-3 ion transport by occluding the cytosolic chloride entry pathway. Binding of the TMEM9 C-terminus to ClC-3 requires the phosphoinositide PI(3,5)P_2 that is sandwiched between both proteins. CLC/TMEM9 function may be regulated by phosphorylation of interacting residues and by modulating PI(3,5)P2 levels.