Structural basis of function and regulation of endolysosomal TRPML channels

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The mucolipin transient receptor potential (TRP) channels (TRPML1-3) localize primarily in endosomes and lysosomes. They conduct Ca^{2+} and Na^{+} currents from the lumen to the cytoplasm and play important roles in the endocytic pathway. Mutations of TRPML1 cause mucolipidosis type IV (ML4), a rare but severe lysosomal storage disorder with cognitive, linguistic, visual and motor deficits. Dysfunction of TRPML3 causes deafness and pigmentation defects in mice. The activities of TRPML channels are regulated by endolysosomal Na^{+}, Ca^{2+}, pH and PIP_2. TRPML subunits have a unique linker, the I-II linker, between the first two transmembrane segments. This linker accounts for more than one-third of the subunits' length and harbors three ML4-causing single amino acid missense mutations, suggestive of its functional importance. To better understand the molecular mechanisms of TRPML channel activation, permeation and regulation, we determined high-resolution structures of an isolated I-II linker and a full-length TRPML channel and carried out structure-guided functional studies. We obtained crystal structures of the TRPML1 I-II linker at different pH values (4.5, 6.0 and 7.5) that correspond to the pH in lysosomes, endosomes and the extracellular milieu, with resolutions of 2.3 or 2.4 Å. The structures at different pH conditions are virtually identical. The linker adopts a new structural fold and forms a tetramer with a highly electronegative central pore lined by a novel luminal pore-loop. Mutagenesis studies show that Ca^{2+} and H^{+} interact with the luminal pore-loop to exert physiologically important regulation. The MLIV-causing mutations disrupt the luminal domain structure and cause TRPML1 mislocalization. We also solved cryo-EM structures of full length human TRPML3 in the apo, agonist-bound, and low-pH-inhibited states, with resolutions of 4.06, 3.62 and 4.65 Å, respectively. The agonist ML-SA1 binds between S5 and S6 and opens an S6 gate. The selectivity filter is lined by a combination of carboxylate side-chains and backbone carbonyls, explaining nonselective monovalent cation and Ca^{2+} permeability. The I-II linker has a structure highly similar to that of the TRPML1 I-II linker and constitutes a polycystin-mucolipin domain (PMD) on the luminal side of the channel. The PMD forms a luminal cap atop the transmembrane domain. S1 extends into PMD and forms a 'gating rod' that connects directly to the luminal pore-loop, which differs structurally from the luminal pore-loop of TRPML1 and undergoes dramatic conformational changes in response to low luminal pH. S2 extends intracellularly and interacts with several intracellular regions to form a 'gating knob'. These unique structural features, combined with electrophysiological studies, reveal a new allosteric mechanism whereby luminal Na^{+}, pH and PIP_2 regulate TRPML3 by changing S1 and S2 conformations. Our studies reveal unique and interesting structural designs and provide blueprints for understanding and exploring TRPML channel function, regulation, pathogenesis and therapeutic strategies.