CRYOEM ANALYSIS OF GATING DYNAMICS IN MAMMALIAN BESTROPHINS

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The bestrophin family of calcium-activated chloride channels are integral to eye physiology. Normal function of the human Best1 channel (hBest1) is required for the maintenance of retinal function, and over 250 mutations in the BEST1 gene lead to a collection of related eye diseases broadly termed "bestrophinopathies." The Best2 homolog is expressed in the basolateral membrane of the nonpigmented epithelial cells of the ciliary body, where it has been suggested to play a key role in aqueous humor production through its calcium-dependent activity. Structure-function studies on bestrophins have revealed their overall architecture and established the critical function of two narrow occlusions to the anion conduction pathway, the neck and the aperture. Despite previous studies, questions remain regarding the molecular mechanisms by which mammalian bestrophins maintain their anion selectivity while gating to facilitate ion flow through the channel and across the plasma membrane. This study utilized single-particle cryogenic electron microscopy (CryoEM) to assess conformational dynamics associated with gating in mammalian bestrophin channels. The structures of mammalian bestrophins in various gating states were solved at resolutions ranging from 1.9 to 3.0 Å under different calcium concentrations, providing the molecular basis for proposed gating models. These gating models were then tested by electrophysiological analysis of rationally designed mutants. Our studies provide significant insight into the molecular underpinnings of the biomedically-relevant mammalian bestrophin channels and shed new light into gating dynamics and conserved mechanisms of anion selectivity.