Cryo-EM structure of human Aquaporin 8 and a possible non-histidine dependent regulatory mechanism

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Aquaporins (AQPs) are water channels able to facilitate the flux of water and other small solutes across the plasma membrane and/or mitochondrial membrane of the cell[1]. AQP8 is distributed across human tissues, particularly in the colon and the epithelium lining the gut and has been implicated in various gut disorders such as inflammatory bowel disease and colon cancer [2,3]. The protein is located in both plasma and inner-mitochondrial membranes, and is postulated to facilitate the flux of water, urea, NH_3, and H_2O_2.

Here, we report the structure of human AQP8, determined using cryo-EM at 2.4 Å overall resolution. We show that AQP8 holds the classical AQP-fold of six membrane-spanning helices (TM1-6), and pinpoint waters along the conducting pores that are visible in the cryo-EM density. Pore analysis reveals a potential regulatory site at a conserved histidine, restricting the conduit near the interface towards the 'inside'. Comparisons to the open structure of the homologous AQP *At*TiP2;1 however suggest an alternative, non-histidine dependent gating mechanism, based on shifts of an adjacent valine of TM4. The position of this amino acid may in turn be orchestrated by a salt -bridge network on the 'inside'-facing end of TM4, sensitive to changes in ion concentrations and/or pH. The setup may perceive environmental changes of the inside of mitochondria, to assist regulating metabolically critical processes.

Conversely, our functional data suggest the narrowing has little impact on the solute specificity for urea and H_2O_2, and rather that this is established by the so-called Ar/R-region, and a critical function of a phenylalanine that extends this filter. Moreover, our structure reveals the presence of a lipid blocking the central pore, presumably precluding passage of small compounds via this pathway and we speculate this may be generally applicable for AQPs. Collectively, our work thus provides unique molecular insights into the molecular determinants of human water homeostasis.

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