Annotated Bibliography: Aquaporins

I. Articles

1. Bao, F., Chen, M., Zhang, Y., and Zhao, Z. (2010). Hypoalgesia in mice lacking aquaporin-4 water channels. *Brain Research Bulletin* 83, 298–303. doi:10.1016/j.brainresbull.2010.08.015.

This paper elucidates the role of aquaporin-4 channels in pain, using behavioral assays and electrophysiology. The authors first investigate motor function and coordination in the mice to determine if behavioral differences may be due to differences in movement rather than pain perception. Behavioral tests for pain sensitivity include a hind-paw radiant heat test, tail flick test, hot plate test, von Frey test, and a Formalin test. The first 3 assays test for heat pain, the fourth tests for mechanical pain, and the last tests for chemical pain. The behavioral tests were followed by extracellular recordings in the superficial dorsal horn of the spinal cord. Overall, the authors conclude that aquaporin-4 knockout mice have altered nociceptive processing, as indicated by increased latency to withdraw from heat, and reduced number of neurons that responded to nociceptive stimuli.

2. Brown, D. (2017). The Discovery of Water Channels (Aquaporins). *Ann Nutr Metab* 70, 37–42. doi:10.1159/000463061.

This paper outlines the significance, function, models, discovery, diversity, and pathophysiology of aquaporins. Written at about an undergraduate level, this paper is a nice place to start reading about aquaporins to gain a better understanding of the basics and history, and provides useful references.

3. de Bellis, M., Cibelli, A., Mola, M. G., Pisani, F., Barile, B., Mastrodonato, M., et al. (2021). Orthogonal arrays of particle assembly are essential for normal aquaporin-4 expression level in the brain. *Glia* 69, 473–488. doi:10.1002/glia.23909.

de Bellis *et al*. utilized a new mouse model, generated using CRISPR/Cas-9, to study orthogonal arrays of particles (OAP) assembly *in vivo*. Aquaporin-4 often forms orthogonal arrays of particles made up of two isoforms: M1 and M23. The function of these arrays is largely unknown so this mouse model, which lacks the M23 isoform, is a key tool to use in OAP research. The authors found that the astrocytes of mice lacking OAPs had reduced aquaporin-4 expression and reduced density of aquaporin-4 aggregates. Future studies using the OAP deficient mice could investigate behavioral consequences of the lack of OAPs in astrocytes.

4. Canessa Fortuna, A., Zerbetto De Palma, G., Aliperti Car, L., Armentia, L., Vitali, V., Zeida, A., et al. (2019). Gating in plant plasma membrane aquaporins: the involvement of leucine in the formation of a pore constriction in the closed state. *FEBS J* 286, 3473–3487. doi:10.1111/febs.14922.

This paper describes gating in plant aquaporins, which play a crucial role of retaining moisture and protection from dehydration. The authors use sequence analysis, simulation, and oocyte water transport experiments to understand how a leucine (nonpolar, amino group, carboxylic acid group) residue blocks water transport in response to pH changes. In a mutant channel, with the leucine residue replaced by an alanine (amino group, carboxylic acid group, shorter carbon chain than leucine) residue, cell swelling was not prevented at lower pH where a wildtype channel would be inhibited. They conclude that the leucine residue gates the pore at the cytoplasmic entrance point, and is sensitive to pH modulation.

5. Denker, B. M., Smith, B. L., Kuhajda, F. P., and Agre, P. (1988). Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. *Journal of Biological Chemistry* 263, 15634–15642. doi:10.1016/S0021-9258(19)37635-5.

This manuscript from the Agre lab describes the identification and purification of a 28 kDa protein from both erythrocytes and renal tubules. This is a major publication regarding aquaporins before they had been identified as water channels. Cellular and molecular techniques used include immunoprecipitation, SDS-PAGE, Western blotting, antibody preparation, and histology. The novel 28 kDa protein was determined to be an integral membrane protein since it was present after peripheral proteins were removed. The protein is also glycosylated, and exists as many bands (indicating multimers) in blots. Localization of the protein to red blood cell membranes and kidney tubules was an early hint that the protein likely played a role in water movement. Dr. Peter Agre won the Nobel Prize in Chemistry in 2003 for the discovery of aquaporins (shared with Dr. Roderick MacKinnon).

6. Hub, J. S., and de Groot, B. L. (2008). Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proceedings of the National Academy of Sciences* 105, 1198–1203. doi:10.1073/pnas.0707662104.

Hub and de Groot take a computational approach to understand the selectivity of aquaporins and aquaglyceroporins. Using simulations and calculations of force for solutes crossing through an aquaporin (water selective), aquaglyceroporin (water, glycerin, and other solutes can pass), or the lipid bilayer, this paper illustrates interactions that contribute to selectivity. One location that is a barrier is the ar/R region (aromatic-arginine). In an aquaglyceroporin the barrier at this region is less than the barrier in an aquaporin-1, suggesting this site is partially responsible for the reduced selectivity of the aquaglyceroporin. This region is wider and more hydrophobic compared to aquaporins, enabling the aquaglyceroporin to have permeability for small solutes.

7. **Lu, D. C., Zhang, H., Zador, Z., and Verkman, A. S. (2008) Impaired olfaction in mice lacking aquaporin-4 water channels. *The FASEB Journal*, 8.**

AQP-4 is highly expressed in the supporting cells within the main olfactory epithelium, likely playing a role in maintaining the hydrated mucus layer in the nasal cavity. Despite the epithelium appearing normal, AQP-4 knockout mice had reduced water permeability, and impaired performance in both a buried food test and an olfactory maze. Electroolfactogram recordings revealed that knockout mice had reduced amplitude in response to common odorants including acetophenone, 1-butanol, triethylamine, thioglycolic acid, and 4-fluorobenzaldehyde. Finally, the authors hypothesize that the olfactory phenotype in the knockout mice is mediated by disrupted K^+ buffering involving AQP-4.

8. Madeira, A., Moura, T. F., and Soveral, G. (2016). Detecting Aquaporin Function and Regulation. *Front. Chem.* 4. doi:10.3389/fchem.2016.00003.

This mini-review is an awesome resource for laboratory techniques and assays, and strategies for therapeutic potential of aquaporins. Cellular models, permeability assays, epithelial assays, osmotic swelling assays, microscopy techniques, stopped-flow spectroscopy, computational methods, inhibition, and short-term regulation are all described in detail. I found this resource particularly useful to understand techniques we had not covered in class such as swelling assays and stopped-flow spectroscopy.

9. Newby, Z. E. R., O'Connell III, J., Robles-Colmenares, Y., Khademi, S., Miercke, L. J., and Stroud, R. M. (2008). Crystal structure of the aquaglyceroporin PfAQP from the malarial parasite Plasmodium falciparum. *Nat Struct Mol Biol* 15, 619–625. doi:10.1038/nsmb.1431.

This paper describes the crystal structure of an aquaglyceroporin from a parasite (PfAQP) at 2.05 Angstrom resolution. The channel was crystallized as a tetramer. The channel exhibits four-fold symmetry and the central pore in the middle of the 4 monomers is blocked by four tyrosine residues. The pore is shaped like an hourglass and is as narrow as 3 Angstroms. Interestingly, PfAQP in proteoliposomes has conductance of glycerol comparable to that of water.

10. Solbu, T. T., and Holen, T. (2012). Aquaporin Pathways and Mucin Secretion of Bowman's Glands Might Protect the Olfactory Mucosa. *Chemical Senses* 37, 35–46. doi:10.1093/chemse/bjr063.

The olfactory epithelium is unique since primary sensory neurons contact the external environment. These neurons and other cells within the epithelium are protected from dehydration by water regulation and mucous secretion. This group investigated distribution of water channels throughout the main olfactory epithelium of wildtype and AQP-4 knockout mice. Expression of AQP-5 was present on Bowman's gland ducts at the surface of the epithelium. This is not a surprising finding, yet mucous production in the epithelium was not yet fully understood. The olfactory epithelium is protected by mucous, which is produced by Bowman's glands. AQP-4 knockout mice have poor olfaction, yet had intact Bowman's glands with no clear defects. AQP-

1 expression was localized to venules near the epithelium. This study begins to elucidate the role of aquaporins in olfactory epithelial hydration and mucous.

11. Savage, D. F., and Stroud, R. M. (2007). Structural Basis of Aquaporin Inhibition by Mercury. *Journal of Molecular Biology* 368, 607–617. doi:10.1016/j.jmb.2007.02.070.

Mercury is a known inhibitor of some AQPs, yet the mechanism of inhibition is not well understood. This paper describes the structural basis of mercury binding of a mutant of aquaporin AQP-Z. It was known that the mercury-sensitive residues were cysteine 189 of aquaporin-1 and threonine 183 in AQP-Z. The mutant channel had the Cys 189 of AQP-1 but lacked all other cysteine residues (replaced by serine). The AQP channel was found to bind mercury in two places: in the pore and interstitially. The authors hypothesized that the pore was blocked sterically by the mercury within the pore; they proceeded with a mutant channel that only binds mercury at the pore. X-ray crystallization of this channel reveal four mercury atoms within the pore near the arginine-proline-alanine motifs. The first mercury atom is bound within the pore, and the first atom may promote binding by a second mercury atom in one of the other three positions available. Savage and Stroud's series of experiments that provide the basis for future studies to discover other inhibitors with potential therapeutic use.

12. Yan, Z.-J., Wang, D., Ye, Z., Fan, T., Wu, G., Deng, L., et al. (2020). Artificial Aquaporin That Restores Wound Healing of Impaired Cells. *J. Am. Chem. Soc.* 142, 15638–15643. doi:10.1021/jacs.0c00601.

Prior to this publication artificial water channel research has progressed, leading to construction of a synthetic water channel that works similar to AQPs in cell membranes. The key feature contributing to water permeability of aquaporins is the hourglass pore region, which uses ionic interactions and size constriction to limit passage of just water. This group designed three channels mimicking these properties of water channels, with different charged residues at the channel pore entrance. The channel 1 (+ charges at both pore entrances) was used through the series of experiments. Channel 1 spontaneously incorporated into lipid bilayers, was able to restore mercury-induced impairment of AQP, exhibited ion restriction similar to AQP, and accelerated wound healing in cells with nonfunctional AQPs. Overall, this series of experiments show the potential of channel 1, an artificial water channel, to work similarly to water channels. This has therapeutic potential to restore water channel function in cells with impaired AQPs.

II. Websites

1. https://www.uniprot.org/uniprot/P29972

UniProt has pages for various aquaporin genes. This page is for the human aquaporin-1 gene and has extensive information about function, names/taxonomy, cellular localization, post-translational modifications, tissue expression, interactions with other proteins, structure, amino acid sequence, and more.

2. **https://www.genenames.org/data/genegroup/#!/group/305**

Gene names is part of the Human Gene Nomenclature Committee (HGNC) and has information about the aquaporin genes including abbreviations, any other names of the channel, previous symbols, alternative abbreviations, and chromosome locations.

3. **https://flybase.org/reports/FBgg0000645.html**

FlyBase is a very useful resource for information about genes in *Drosophila* including gene ontology terms and related gene groups.

4. **https://reactome.org/content/detail/R-HSA-432047**

This reactome page shows pathways of passive transport by human aquaporins to pass water, glycerol, or urea in the cell membrane, and anions in transport vesicles.

5. **https://www.3dmoleculardesigns.com/Teacher-Resources/Aquaporin-Mini-Model/Animations-and-Videos.htm**

This website has 3-dimensional model of aquaporin that is animated to show water selectivity and how the oxygen atom in water interacts with asparagine residues to produce this selectivity.

6. **https://www.wikidoc.org/index.php/Aquaporin**

WikiDoc is a useful starting point when looking for information about aquaporins. This website has information about function, discovery structure, aquaporins in mammals, aquaporins in plants, and aquaporins and disease states. In addition to content provided on this webpage, the references provide basic papers to read for further information.

7. **https://proteopedia.org/wiki/index.php/Aquaporin-1**

Proteopedia is Wikipedia for proteins! The page on aquaporin-1 details its discovery, brief details, structure, and regulation.

8. **https://www.genecards.org/cgi-bin/carddisp.pl?gene=AQP3**

GeneCards is a human gene database with information specific genes such as associated promoters and enhancers (for AQP3 on this specific page), size/distance, transcription factor binding sites, and gene targets.

9. **https://atlasgeneticsoncology.org/Genes/GC_AQP1.html**

The Atlas of Genetics and Cytogenetics in Oncology and Haematology (updated in February of 2019) is a website that houses information about aquaporin-1 DNA, protein, and its implication

with different types of cancer. Details include alternative splicing forms, expression locations, epigenetic mutations, an in-depth PubMed bibliography, and references to dozens of other databases that have pages on AQP-1.

10. **https://www.nobelprize.org/prizes/chemistry/2003/agre/lecture/**

The Nobel Prize website is a useful resource as it has biographical information about Laureates, text of their lecture, and even videos of their lecture dating back to 1973. Dr. Peter Agre was awarded the Nobel Prize for Chemistry in 2003 for his work on the discovery of aquaporins. He shared this award with Dr. Roderick MacKinnon for his work on potassium channel structure.