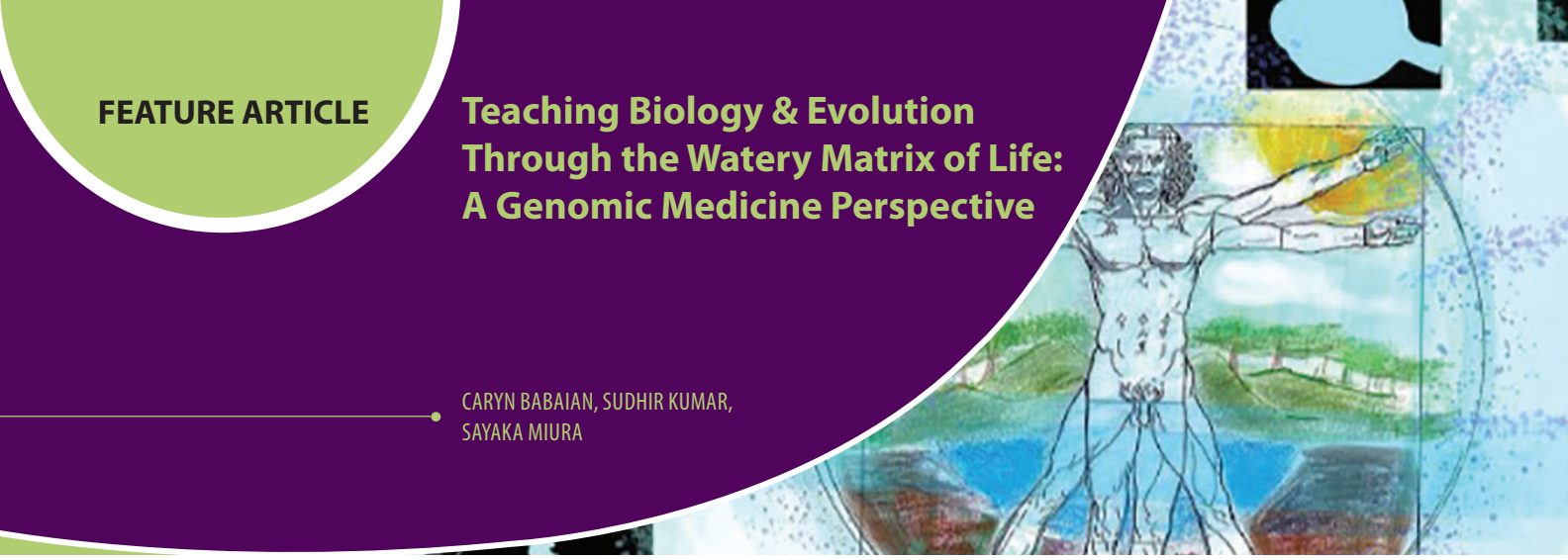
FEATURE ARTICLE

Teaching Biology & Evolution Through the Watery Matrix of Life: A Genomic Medicine Perspective



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Abstract

Water is one of the most common molecules in the universe. Water is polarized, but it has many states besides the normal tetrahedron depicted in standard biology texts. Water is also the most ubiquitous molecule on Earth, the universal solvent. It is the internal and external habitat of cells. Ecologically, water is contiguous with life and the chemistry it nourishes. Water merges with everything from DNA to itself in the vast ocean; it is a constant molecule, and it does not change—or does it? Water, is the planet's unwavering, flowing, but fixed liquid substrate, and it has an elemental and evolutionary story to tell. Water can independently regulate solute transport, entangling with cell proteins to create the aqueous conditions that support life metabolisms and the evolution of other molecules. Water dynamics are rarely mentioned in standard biology discussions, even though biomolecules are strongly influenced by the hydration shells around them. For water to affiliate with all things living requires specialized entry and exiting of water, achievable by a ubiquitous channel protein called an aquaporin. In this article, we will explore water's often neglected complex relationship with all things biological from an aquaporin perspective. The aquaporin family of proteins is ancient and spans the tree of life in archaea, bacteria, protozoa, fungi, plants, animals, and viruses. From DNA to osmoregulation, aquaporins literally channel the water molecule through geological time. We will also explore the bigger picture of the aquaporin as a teaching tool for evolution. Through the genomic medicine paradigm, we examine diseases that manifest from defective aquaporins. From a visual and arts perspective, we reframe biological processes in the light of the most abundant but nominally understood molecule on Earth: water.

○ Introduction

The Aquaporin Teaching Hypothesis

Few concepts unite biological systems like evolution. It is frequently said that "nothing makes sense in biology, except in the light of evolution." This quote from Dobzhansky has become iconic, and while true, biology may make even more of a palpable sense in the light of water. In fact, it's not just biology but evolution itself that could be illuminated through water's diverse conduit, the aquaporin. If we shift the paradigm we can view water's taciturn evolutionary role with life processes through an *Aquaporin teaching hypothesis*. For almost all metabolisms, reactions, divisions, replications, mutations, and conformational changes to occur in a living cell, water is required. Water is biologically ubiquitous, but still underrepresented in conceptualizing the molecular world of biology.

From viruses to plants, to fungi, to humans, aquaporins are the interface between watery ecologies inside and outside the cell.

For the earliest metabolisms, the first nucleic acid synthesis needed regulated water concentrations. Once cell membranes are formed, an adjusting conduit from outside to inside becomes a necessity. How and when did this first occur? Can aquaporins help create an evolutionary narrative in biology? Aquaporins were discovered toward the end of the twentieth century. Since then, an increasing number of genome and transcriptome sequencing projects have produced thousands of orthologous channels across the tree of life from archaea through diverse eukaryotic cells, encompassing all the domains of life, including non-cellular viruses (Finn, 2015). From viruses to plants, to fungi, to humans, aquaporins are the interface between watery ecologies inside and outside the cell. From molecular associations to infectious disease transmission, to biomolecule fundamental function, to massive self-associations in aquatic ecosystems, water is the adaptable constant in life-shaping moment-by-moment homeostasis through the longest geological time frame. Most biological processes occur in an aqueous environment. To achieve a cell's aqueous environment, water must be present. The facilitator between water's journeys from outside to inside is the transmembrane amphipathic aquaporin channel protein and its associated membrane, also referred to as a "water channel." This dynamic, interactive interfacing channel is the obligatory accomplice that proceeds everything from larger system metabolisms to DNA stability. Since water is the major prerequisite for life, water is an evolutionary molecule, one that contributes to the evolutionary process, and the aquaporin is the phenotypic byproduct of change reflecting water's sweep into and across all lineages in the tree of Life. Students can learn about water and its cellular connections to life through the art and drawing processes presented in this paper.

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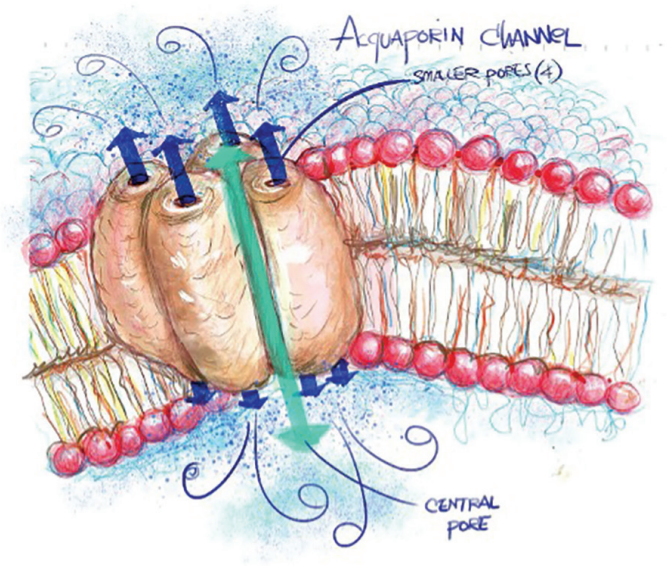

Figure 1. AQP channel with four subunits, arrows indicate movement of water.

The AQP family of proteins passively and selectively shuttle water and other small molecules into and out of cells (Figure 1). They can be divided into two broad categories: the AQP water-only aquaporins and the AQP glycerol aquaporins (King, 2004). Aquaporins are the foundation of homeostasis through osmoregulation and contribute to the overall shape, metabolism, reproduction, biological recognition, and energy states of cells (Fox, 2017). The discovery of aquaporins late in the twentieth century on the surface of red blood cells resulted in a small paradigm shift regarding membrane permeability, one most students never encounter in biology class, imagining that water moves through membranes only via osmosis. The standard discussion on membrane permeability normally does not connect to evolution or the tree of Life, but through aquaporins, a richer, more complex view of evolving cells and the tree of life is possible. Pressured by the necessity of water-biomolecule interfaces leading to increasingly complex molecular relationships, aquaporins have come to occupy niches in physiology, enabling life processes to happen in varying and specific ways as there are many differently sized water channels with a variety of functions. These nuanced requisite reaction interfaces allow organisms to use water and solutes to attune and become one with the place they inhabit, whether terrestrial or aquatic.

Water-specific proteins are typically discussed only in the osmoregulation of the kidneys and related structures, but AQPs are found not only throughout the tree of Life but also throughout the human body (Finn, 2004). From an evolutionary perspective, water is a constant and aquaporins may have been the first communicating conduits of cells such as the hypothetical and elusive LUCA. In an ecological sense, aquaporins through distinct cellular spaces, give organisms physiological plasticity and specific adaptations on an evolving watery planet. Water is involved in DNA-ligand binding, which helps drive evolutionary specializations. In prokaryotes such as lactic acid bacteria, AQPs assist with the fermentation process (Tong, 2019). Water moves through AQPs in protozoans, such as the amoeba and paramecium, and affiliates with specific and specialized vacuoles. In fungi, specific aquaporins are essential for fruiting body emergence and gene expression for spore activation and dispersal (Nehls, 2014). In non-vascular plants such as bryophytes, aquaporins facilitate desiccation intolerance (Oliver, 2008). In ferns, water provides the medium for the movement of sperm and aquaporins mediate ion compositions in varying soil types affecting fern reproduction (Lin, 2021), and in higher plants xylem and phloem are regulated by aquaporins that contribute to the viscosity of dissolved sugars. In our own bodies, aquaporins are found in the membranes of red blood cells, adapting their shape to the viscosity and solute concentration of blood and in the kidneys they help create a salty niche for regulating water. Diseases and mutations in aquaporins can reveal their widespread function throughout the human body providing a more comprehensive view of our intimate relationship with the water molecule. It becomes apparent that across the tree of life, aquaporin's channel the planet's water.

○ Water Across the Tree of Life, from Snowflakes to Raindrops to DNA

Most students are introduced to early life and macroevolution through concepts of a reducing atmosphere on Earth, tumultuous oceans, lightning storms, Miller, and Urey, and ultimately the formation of small biomolecules where somehow electric currents "zapped" life into existence. Electric interactions played a fundamental role for the properties of hydrated bio interfaces where water molecules interact through electric fields. Water is both the source of those charged interfaces and the constituents of them (Shweta, 2018). Bio interfaces through electrostatics and Van der Waal forces are foundational in membrane formation, metabolisms, and DNA binding (Nguyen, 2008). DNA is embedded in a water shell and its shape and function are modeled by the ions around it. "*Water and ions control chemical processes in biological systems thereby directly and indirectly facilitating the functions of biomolecules such as nucleic acids*" (Shweta, 2018; Ball, 2008). Water not only created the conditions for life to emerge and for DNA to replicate, but it could form extensive networks because of its bonding, form crystals to surface tension, and link up with DNA to encourage substrate bonding.

Water is indeed an unusual molecule; it has not changed in billions of years and yet its polarity allows it to dissolve other molecules and dissociate into hydronium and hydroxyl ions generating the pH scale that all students encounter in basic chemistry. Dissolved minerals from rocks created the salty oceans, which flow into the unsalted regions of the hydrological cycle to freshwater basins, through this cycle water becomes part of our physiology and part of our DNA. Genes and chromosomes do not function without water, enzymes don't work without water, blood doesn't exist without water, oxygen isn't generated if water isn't present, and in water's absence, a wide range of desiccation-based adaptive strategies fail to evolve. Is it possible that in teaching evolution we might consider that water co-evolves with proteins, nucleic acids, and cells? Like an enzyme water interfaces temporarily and yet remains unchanged. From an aquaporin perspective water soon becomes the main character in the story of evolution instead of a backdrop and something that can give students a bigger eco-evolutionary picture. *"For long, water was considered as rather a passive background matrix on which only suitable biomolecular structures were thought to permit the mammoth of biological functions"* (Gerstein and Levitt, 1998).

Aquaporin evolution would have to have arisen very early as an absolute necessity of cells to regulate water in specialized spaces to perform its rapid, multitude of interactions. Water contains within its fluid structure a slightly electronegative oxygen and two slightly

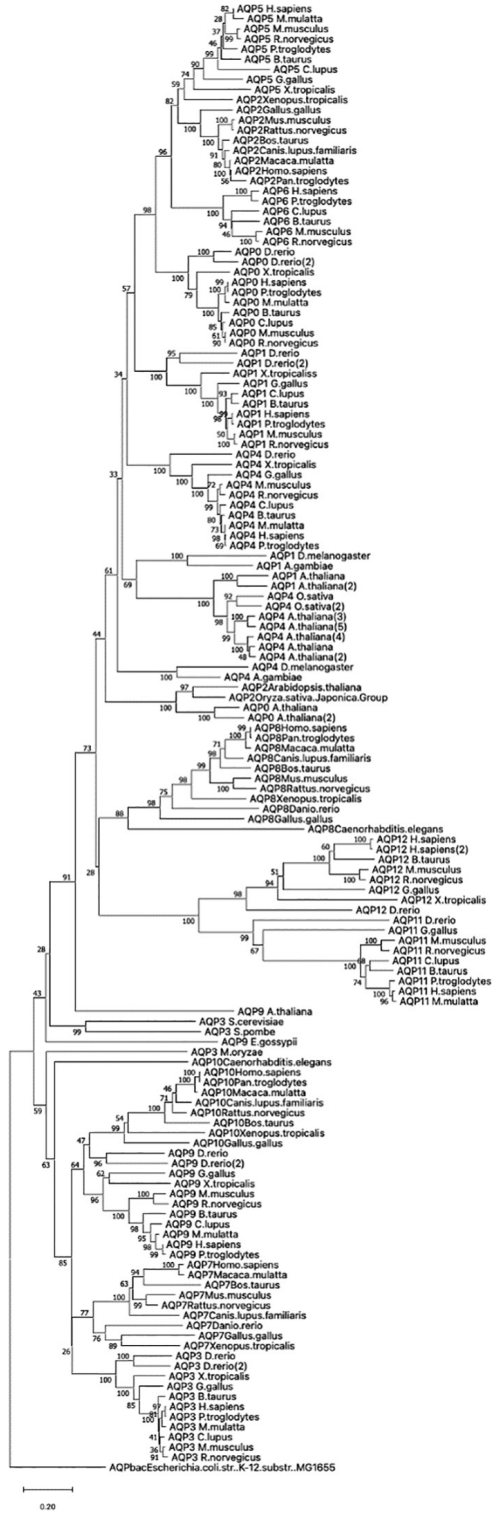

Figure 2. An evolutionary tree of Aquaporin gene family reconstructed using the MEGA software. Aquaporin protein sequences were acquired from the HomoloGene database (https://www.ncbi.nlm.nih.gov/homologene/). They were aligned utilizing CLUSTALW in MEGA, employing the default parameters. The neighbor-joining method was used to build the phylogeny based a Poisson model, with 500 bootstrap replicates used to assess the robustness of the inference. The resulting phylogenetic tree was rooted with a sequence from *Escherichia coli*. Step-by-step instructions are available from igem.temple.edu/genomicmed.

electropositive hydrogens. This is enough to provide a jump start to ion exchange and interfaces the kind that fuel molecular diversity and sophistication. This ubiquitous nature of aquaporins across geological time is visible in the AQP tree (Figure 2). The tree is rooted in a prokaryote (*E.Coli*). Teachers can have students make the tree or they can just examine the various species on the tree and look at relatedness. Phylogenetic trees using such a ubiquitous protein channel give an automatic "big picture" view to evolution and the shared aquaporin protein. A fundamental question to present to students might be "How does the phylogenetic tree make sense of relatedness across the tree of life?" or "Why are aquaporin's found in almost every living thing and in every part of our body?" "Big" questions can reframe the diverse roles of aquaporins and the absolute necessity of water. (Please see our website at the end for activities.)

○ Aquaporins: Current Background

When did AQPs come into the watery scene of Earth? AQP ancestors started in prokaryotes by the duplication of genes that then diversified to eukaryotic cells evolving specific carboxyl terminal functional groups that can be traced to plant and fungal lineages (Ishibashi, 2017). Whole gene duplications and HGT played a role in AQPs spreading through invertebrate and vertebrate linages. Viruses probably acquired the AQP through HGT (Ishibashi, 2020). The necessity of aquaporins throughout the tree of life is clear, living cells and even viruses need to channel water to grow, move, replicate, and evolve. Phylogenetically, aquaporins belong to the major intrinsic protein (MIP) family that is composed of more than 1700 integral membrane proteins (Baiges, 2002). Biochemical analysis of aquaporins indicates a fundamental monomeric structure with a signature amino acid sequence of Asn-Pro-Ala and an amphipathic channel lining. Aquaporins are situated in the membrane as tetramers with each monomer component containing a channel for water, glycerol, small ions, and even simple gases such as carbon dioxide (Kruse, 2006) (see Figure 1). Aquaporins, like all biological molecules, wear many hats and not surprisingly since they are found in so many types of cells and almost all tissues and organ systems of the body. Since sequencing methods have improved radically since the 1990s, a huge diversity of aquaporins have been discovered. Regardless of remarkable structural similarity between all aquaporin channels, it is also not surprising that they contain refined and slight changes to their structures to adapt and adjust water and affiliates to unique cell and tissue requirements as there are 13 known AQP isoforms in humans (Zhu 2016). A gating mechanism strategy employed by aquaporins is a narrowing of their channel, which sits above the bilayer of the plasma membrane, along with a highly conserved arginine residue in a fixed position with a strong negative charge acting as a second gating mechanism (Sachdeva, 2014). Aquaporin permeability can be altered by pH, temperature, concentration, and a host of other variables. The aquaporin family tree roughly correlates with their permeability characteristics indicating that their early transport function was most likely involved in cell evolution and regulation of basic activities (Finn, 2015). Aquaporins can also be very specialized, in some organisms such as mosquitos they can transport sugars such as trehalose, while a mammalian aquaporin AQPb0 ortholog may function as a cell adhesion molecule (Kumari, 2013). Despite being labeled as water channels, aquaporins can accommodate a large diversity of molecules, where and when such diverse lineages and function arose through time is a complex and unresolved subject in aquaporin

biology and too tangled for our purposes, but it reveals that there are many unresolved questions about both water and their AQP channels including how water contributed to AQP evolution. We want to explore the potential of aquaporins to inspire critical and divergent thinking in students regarding evolutionary processes and for students to see the connection between the planet's hydrological cycle, evolution, and physiology.

○ Water and AQPs at the Molecular/ Evolutionary Interface

Depictions and descriptions of DNA replication and repair, mutational events and conceptual models and mechanisms of evolutionary and metabolic change almost never include water, and it's easy for students to forget how critical water really is to life because of this absence. For mutations to take place, an aqueous environment is required, the dynamics of such things as DNA repair enzymes require water, but do students actually think about this when studying mutations? In this way aquaporins become the changing, evolving artifact of such dynamics. It might be said that water is essentially the cocreator of the diversity of life on Earth and integral to the molecular evolution hypothesis. Water makes life evolve, it proceeds every reaction and therefore evolution-based processes such as mutations. Water, aside from its osmoregulatory association, participates in decreasing the entropy of a system as it participates in every transitional form through hydration, facilitating conformational change and dynamics such as enzyme and substrate interactions (Davidson, 2013). Students can think about mutational events by hydration shells and by taking a close-up look at how water might regulate such events (see Figure 3). What about the dynamics around the DNA molecule? Can water influence mutations? The rate of evolution? Something students are never taught in units of DNA is that the DNA in their cells exists with vibrational layers of water and that stability of the sugar-phosphate backbone contains what is referred to as "the spine of hydration" (Shweta and Sen, 2018). Many significant facets of DNA's function, that is ligand binding, excision of mismatched bases, and stability, are contingent on water's interface with DNA, which is adjusted and synchronized by aquaporins. Further downstream from DNA's function is the transcriptome and then the proteome, which are further guided by water's availability influencing such critical events as protein folding (Shweta and Sen, 2018).

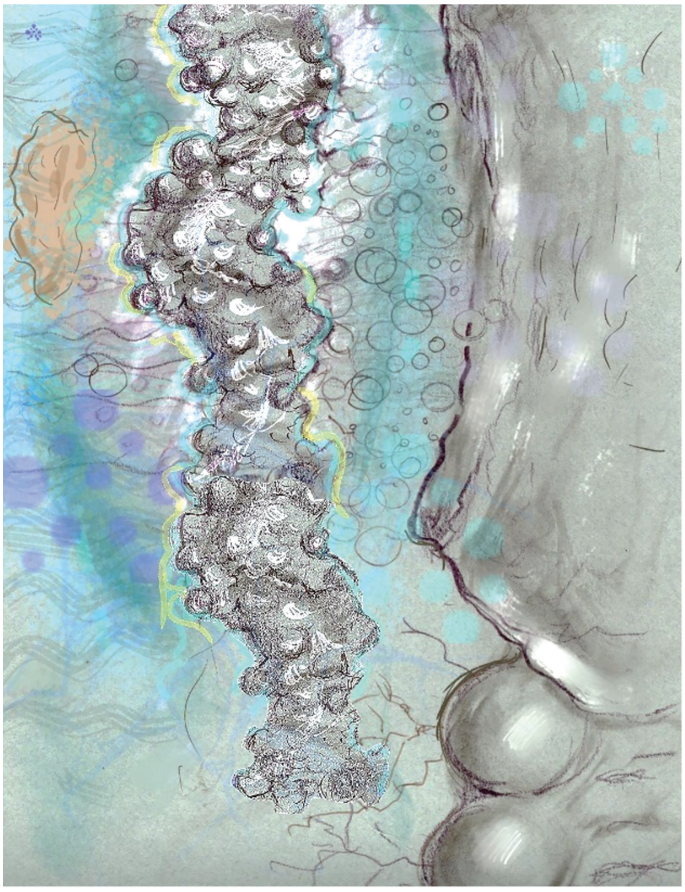

Figure 3. A watery sheath around DNA near an aquaporin.

○ Connecting the Hydrological Cycle to AQPs to the Evolution and Physiology of the Kidney Tubule

Students don't normally connect the hydrological cycle of the planet to their own kidneys and blood, but blood is like a liquid ocean inside our body carrying the ancient solutes in water around to terrestrial environments. Regulating water is often placed under the broad concept of energy gradients with a discussion on osmosis, which does not require energy. Students are given three different environments with three red blood cells. In textbooks the cell's fate in different solutions is visualized as a isotonic, hypertonic, or hypertonic condition. This easy illustration demonstrates solute concentration inside and outside of cells and passive movement of water. Wet labs further enhance the understanding of the effect of water on cells, such as the shrinking or bloating of an elodea leaf. Students are taught with models of salt water, freshwater, and brackish water fish, such as sharks, to illustrate organ systems of vertebrate adaptation to watery and salinity variable environments. This discussion can also move from planarian and flame cells, Malpighian tubules in insects, and ultimately to the mammalian kidney. All these representations of life are linked through the AQP. Like all sequential topics, underlying connectedness, despite being obvious often goes missing. Bridging water and evolution across the tree of life is quite easy if we look at AQPs. Our shifted AQP paradigm focuses on water and aquaporins as the universal drivers of evolution.

○ An Easy AQP Example: The Terrestrial-Aquatic Niche of the Nephron

Some phylogenetic analysis of aquaporins reveals that our mammalian water channels go back to the Devonian period (Finn, 2014). Aquaporins have been used to trace tetrapod evolution through coelacanths, anurans, and Amphibia with mammals retaining the ancestral properties within the aquaporin (Finn, 2014). When students study the comparative anatomy of the osmoregulatory systems they often encounter amphibians who use their skin and kidneys to regulate water. They also encounter a diverse system of waste output from uric acid crystals in reptiles and birds, to urea and ammonia output in fish and mammals. This suggests that aquaporin's evolved gene roles, retaining some functions, losing regions, and localizing expression of specific aquaporins as some survived

extinction and were repurposed for various adaptations. As an example, mammals are the AQP6 aquaporin that was retained from reptiles and functions in the intracellular spaces of podocytes in the glomerulus contributing to acid-base balance and glomerular filtration, but have a reduced function compared with other aquaporins (Nishimura, 2013). For terrestrial animals, retaining water is essential. The evolutionary significance of water conservation is visible in the inherited disease diabetes insipidus, which is a nephrogenic disease caused by mutations in AQP2 or the AVPR2 receptor. Large amounts of urine result, causing rapid dehydration (Grunert, 2020). This unresolved evolution to tetrapod evolution segues into a discussion on the amazing configurations in our own kidneys. The nephron of the kidney is perhaps one of the most relatable models for illustrating the specialized niche of water in cells, the evolution of an entire system around the water molecule and the aquaporin proteins, which facilitate and expand the repertoire of water in its diverse capacity for the orchestration of a broad variety of specific molecules and ions in a specific salinity and niche within an organ. The basic functioning unit of the kidney, the nephron is a fascinating brackish vicinity where a clear divergence of habitats has evolved in one place, similar to a biome that contains both a deciduous forest and a wetland. The kidney highlights a complementary evolution of aquaporins in lieu of water. When the circulation of blood enters the afferent arteriole from the renal artery into the glomerulus inside Bowman's capsule, we get an immediate constriction of the renal arteriole into a bunched-up tuft of capillaries, in that constriction, the plasma of blood becomes what we call filtrate and this is further facilitated by fenestrations and podocytes lining Bowman's capsule. Water thus changes its solute-carrying potential and makes its way into the proximal convoluted tubule. The nephron projects into the medulla of the kidney, an environment where concentrations of salt are maintained to facilitate further regulation of the water content of the body. In the nephron we have two opposing tubules, the proximal descending loop and then the distal ascending loop, in the middle connecting them both, the loop of Henle. The proximal side is rich in aquaporins, the distal side is completely devoid of aquaporins and instead contains transmembrane protein channels, which are possibly the evolutionary great grandchildren of the aquaporin. The proximal tubule carefully adjusts water molecules until the loop of Henle is reached. The hairpin turn of the loop of Henle is ensconced in the salty sands of the medulla, which are maintained by its multiple channel proteins, there are a few aquaporins and a few ion channels, once the loop turns upward toward the distal tubule, and then literally no aquaporins regulating various charged particles such as calcium chloride, sodium, and potassium appear to exist. Water is still flowing through this system, until it makes its way to the collecting duct, all the while the saltiness of the sandy medulla is exerting nuanced controls on water via AQPs. In the collecting duct, if there is too much water, ADH exerts its influence on the kidney by genes turning it up a little or down a little, as ADH is antidiuretic, and is there to modulate loss or retention of water until it becomes urine. In this way, water, salts, and other ions are under continual homeostatic control, varying output all the time so the blood osmolality is maintained. How did aquaporins come to reside on one side and not the other? What drove this unique juxta positioning of proteins in tubules? What fostered the divergence of the aquaporin protein into the same tubule making them dissimilar and distinct, with antagonistic functions? Students can think about what pressures drove the nephron tubules and the surrounding space in the urinary system to evolve.

○ AQPs in Disease: A Genomic Medicine Perspective

AQPs are found throughout the body, anywhere mucus is produced, gas exchange takes place, and water is tightly regulated. The old term for the posterior chamber of the eye, the "aqueous humor" was more descriptive, as AQPs are found in the eye, in the lungs, in the digestive system, in the blood-brain barrier, on erythrocytes, in the cerebellum, in cartilage, in the urinary system, and the list goes on. To address this universality, we have created a landscape image of AQP locations (see Figure 4). Teachers can use this figure to answer students' questions about anatomy location and physiology and then they can move into a discussion on disease. Not surprisingly, many diseases from complex to infectious, to Mendelian condition implicate AQPs. In skin disease, the AQPs are expressed throughout the major divisions of the cutaneous skin, from hypodermis, to dermis, to epidermis. In diseases such as psoriasis, there is implication that keratinocyte physiology may involve aquaporins through a proposed down-regulation and mislocalization of an AQP (Bollag, 2020). Even in melanoma, aquaporins are implicated. An autosomal dominant cataract disease involves an APQ0 mutation (Verkman, 2008). In the neurological conditions involving cerebral spinal fluid, AQPs play a central role in edema, traumatic brain injury, brain tumors, and stroke as they become severely disrupted in the regulation of water channels through cell membranes and the blood brain barrier (Filippidis, 2016). Functionally diverged human aquaporins are involved in a wide variety of non-infectious diseases including renal dysfunction, neurological disorders, epilepsy, obesity, metabolic syndrome, and heart disease. Even in infectious diseases, pathogenic bacteria, fungi, viruses, and parasites can cause systemic infections and sepsis, facilitated by compromised aquaporins (Azad, 2021). Bacterial endotoxins have an effect and can even regulate aquaporin expression (Azad, 2021). The Plasmodium that causes malaria can influence aquaporins in the liver of the host as a host membrane sheaths the parasite when it merges in the cells of the host, the aquaporin in this membrane provides the AQP3 aquaporin that enables the transfer of nutrients from the host to the parasite (Liu, 2016). This would make the aquaporin a target for malaria treatment. In the human body, students can explore the tissue-specific distribution and physiological relevance of aquaporins by looking at a visual map of them. Aquaporins are in the lungs, immunity, wound healing, cell proliferation, and transport of waste. Their global, universal appearance and the appreciation of aquaporins suddenly make water a remarkable valuable biological topic.

AQPs have important roles in the host-pathogen interaction (Azad, 2020). Those roles roles include the homeostatic control of inflammatory processes (Azad, 2020). To explore the physiological and pathophysiological landscape of AQPs, see Table 1.

○ Water as We Age: An Aquaporin Aging Hypothesis?

The control of water homeostasis in living cells is facilitated by aquaporins, which can be extremely selective, but the water in cells expands its repertoire of interactions to all cellular components and, therefore, aquaporins cannot be viewed simply as homeostatic for osmoregulation. For their complex role, aquaporins must be able to exclude hydrogen peroxide and hydronium ions, while

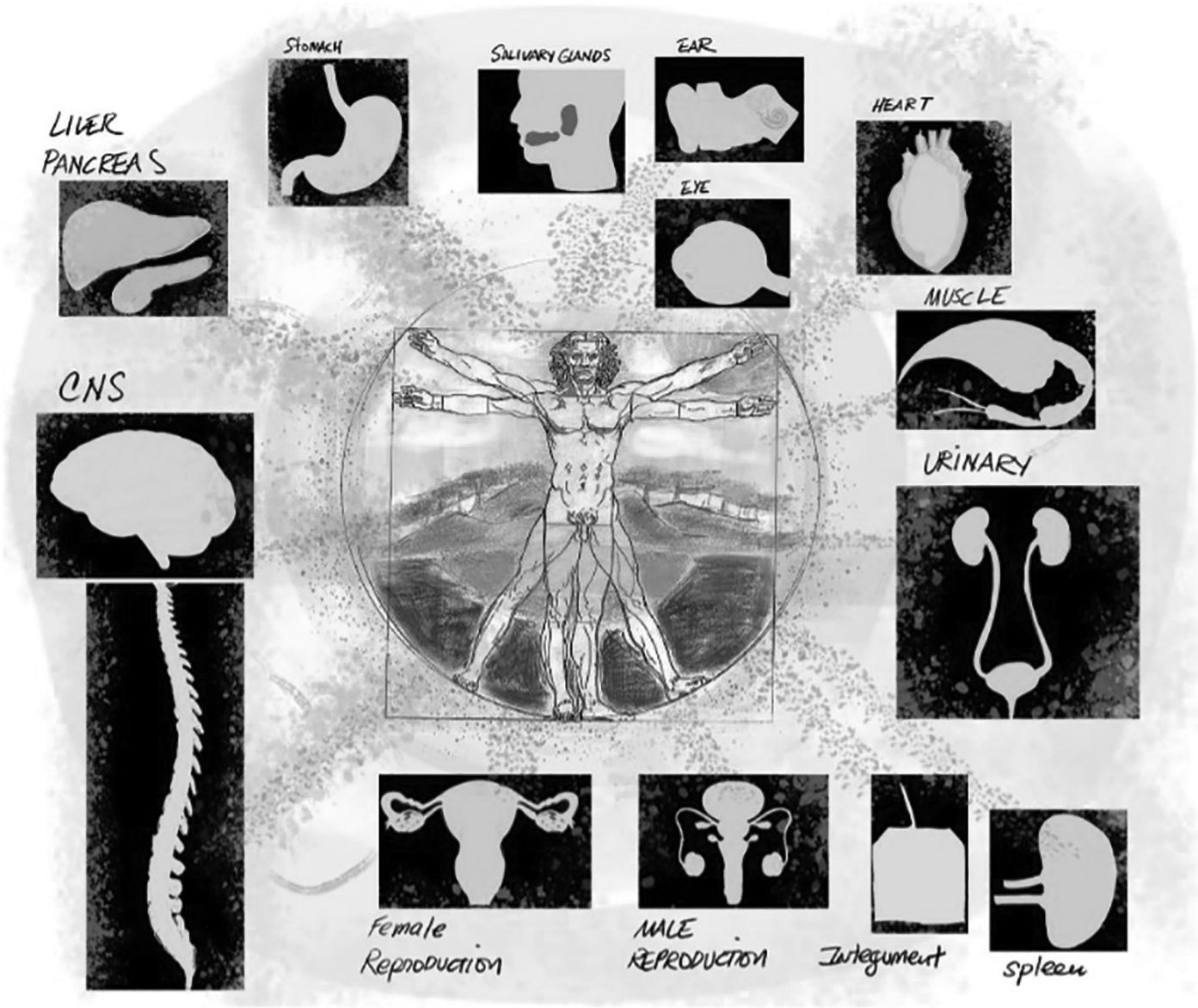

Figure 4. A landscape of the ubiquitous AQP associations with the water cycle depicted behind the Vitruvian man figure. This figure is repeated in black and white in the supplementary section for students to add AQPs to color in, and to label the basic parts of the hydrological cycle.

also preventing the transport of protons. The exclusion of hydrogen peroxide links their functionality to mitochondrial function and to a myriad of other reactions. Proton pumps, chemiosmosis, and energy gradients are, therefore, also dependent on aquaporins (Tesse, 2018). From the GM perspective, mutations in aquaporin genes, as well as the process of aging, reveal the problematic diseases that arise when fundamental channels of homeostasis are disrupted or when senescence of cells occurs. Humans don't desiccate and come to life, but they do dry out as they age. As humans age, aquaporin expression is reduced, fewer and fewer membranes support the functionality of aquaporins. An excellent part of this is in the joints of the body. The plasticity of gene expression and the output of the proteome to make an aquaporin channel, may be timed as well as activated by physical stressors that downturn the expression of the AQP genes through a feedback loop and this might occur throughout the body in eyes, brain, joints, intestines, lungs, and skin (Azad, 2020). Knee cartilage is an aqueous environment filled with synovial fluid. Synovial fluid consists of water, nutrients, and ions that maintain chondrocyte production (Kyung, 2021). Cartilage, a connective tissue, is well hydrated at approximately 75% aqueous water (Kyung, 2021). Aquaporins play a critical role in not only in fibrocartilage functionality but in the inflammatory process. In diseases such as osteo arthritis, gene and protein expression can become profoundly reduced, this prevents chondrocytes from conducting mitosis and differentiating. Responsiveness to reactive oxygen species (ROS) such as hydrogen peroxide can be affected. Expression of aquaporins along with histological images demonstrate that this expression occurs globally, throughout the body cells. Simply put, their surrounding media shrink, and tissues become drier with age (Zhou, 2020). This is another highly visible teaching point that can be immediately be seen in histological images (see Supplementary Material provided with the online

Table 1. Shows a summary of only a few of the known functions of AQP's and related pathophysiology as they occur throughout the body.

	System/Anatomy	AQPs	Function	Associated Disease
Integumentary	Epidermis, dermis, subcutaneous	APQ0, APQ2,3,4,5,6,8,10, & 11	Skin elasticity, cell migration, immune function	Psoriasis, melanoma, wound healing, edema, impaired leukocyte function
Skeletal/Muscle	Striated muscle	APQ 1,3, & 4	Dystrophin associated	
CNS	Brain & spinal cord (blood-brain barrier)	APQ 1,3,4 & 9 (spinal cord) APQ 1 & 4 (brain)	CSF, water homeostasis in the CNS, blood brain barrier metabolism	Edema, hydrocephalus, brain edema, stroke, MD, brain tumors, neurological disorders
Digestive	Mouth, stomach, small intestine, duodenum, jejunum, and ileum, large intestine, liver, pancreas, gallbladder	APQ 1,5, & 8 (salivary glands) APQ 1,3, 5 (oral cavity) APQ1-11 (stomach & small intestine) APQ1,2,3,4,7,8,9,10, 11 (large intestine), APQ1,5,8 (liver & pancreas)	Salivary function, modulation of membrane permeability, mucus production, carbohydrate metabolism, pancreatic exocrine secretions	Sjogren's syndrome, leaky gut, obesity, cholestatic liver disease, liver cancer, IBS
Respiratory	lungs	APQ 3,4,5 (lungs)	AQPs expressed in ciliated columnar cells, support and surround alveoli, pH support, surfactant production, gas diffusion	Lung cancer, asthma, fibrosis, edema, infectious diseases
Circulatory	heart	APQ1,3,4,5,7,9,10, 11 (heart)	Cell migration, pericardial function, inflammatory response, cardiac water homeostasis,	Myocardial edema, arrhythmias, and sudden cardiac death
Urinary	kidney	APQ1,2,3,4,5,6,7,8,11 (kidney)	Glomerular filtration, acid-base metabolism, tubular endocytosis, total water balance	Mutation or functional deficiency of AQP2 leads to severe diabetes insipidus
Reproductive male	Testes, seminal vesicles, vas deferens, epididymis	APQ 0-11	Spermatogenesis, mucus production, seminal fluid	abnormal sperm motility, the abnormal epididymis and infertility seen in cystic fibrosis
Reproductive female	Ovaries, Vagina, uterus, oviduct, placenta, fetal membrane, embryo	APQ 1-12	Mucus production, follicle development, and implantation	Infertility, miscarriage, ovarian cancer
Eye	senses	APQ1,2,3,4,5,7,9,11	AQPs in the eye: water homeostasis, intra ocular pressure. Retina, tear secretion, and lubrication	Autosomal dominant cataracts glaucoma
Ear	Senses	APQ 1,2,3,4,5,6,8,10,11	Fluid balance in semicircular canals, inner ear	Otis media, Meniere's disease, hearing loss
Spleen	Immune	APQ1,3,9	Survival of immune cells, RBC osmolarity	

version of this article). Teachers can also raise questions about water pollutants and recombining chemicals from organophosphates to micro plastics, how do these toxins affect AQP function, what is the implication for the flow of such toxins in the water cycle and for health across the tree of life?

○ Aquaporins, Water Pollution, and Disease

AQPs present another biological relationship between water, the world of modern pollution, and disease. For most people recognizing that water is cyclical is difficult. Water is finite on the planet and most of it is cycled through living things, yet the connection between the modern toxins we produce and disease in our own bodies is a difficult concept to comprehend. Water pollution comes from many sources including air and soil as water cycles through rainfall and ground water. How do conserved, ancient water portals (AQPs) accommodate such a plethora of synthetic toxins? How do these chemicals affiliate with water? How does that affiliation change an aquaporin's response? Since water infiltrates every system of the body, contaminants such as heavy metals can alter AQP function. "AQP4 is a class of aquaporin channels that is mainly expressed in the brain, and its structural changes lead to life-threatening complications such as cardio-respiratory arrest, nephritis, and irreversible brain damage (upon heavy metal exposure) (Maroli, 2019). Nanoparticles used in water treatment have also been shown to have deleterious effects on aquaporins and cause DNA damage (Nallanthighal, 2017). This line of inquiry can lead students into an environmental unit where they can search for studies on the effects of various pollutants in water and their effects on AQPs. Connected to this topic are the yet-undetermined roles of AQPs in cancer, some research suggesting they could be tumor suppressors, other research suggests they might be oncogene triggering.

○ Visualizing and Drawing the Watery Interface

In images of biomolecules and reactions, water is rarely if ever depicted. It is an afterthought once students get passed it in basic chemistry. From excisions of mistaken nucleotides to protein folding, water's associations are not only participatory but pivotal—without them, no such activity would take place (Wspalz, 2009). Like many oversimplifications in science, water is conveniently omitted from visualizations and discussions on evolving proteins, and rarely factored in as a variable in molecular, genomic, and cellular interactions (Dargaville, 2022). Visualizing water's intimate relationship is a challenge but one some students might find rewarding.

Would students think differently about biology if water were consistently represented in biology? Even dehydration-hydration synthesis reactions are not illustrated with the actual water molecule! Would graduate biology students and medical students have a different view of metabolism, ecology, evolution, and the human body if water were a permanent part of biological subjects, as it is in real life? Larger questions arise regarding conceptualizations and models in biology, which omit water from the equation of life. Visualizing the aquaporin's universality. The panorama of paradigms opposing, facilitating, antagonizing, and promoting metabolic innovations within cells because of water is difficult to conceptualize all at once. Asking students to visualize what DNA would look like and how it would function in a dehydrated state versus an aqueous state raises many questions about replication, binding, DNA's shape, and mutations.

○ Conclusion

Water appears simple to students, and it is mentioned only at the beginning of teaching macroevolutionary concepts, but water has many unique configurations, sheathing molecules, adapting and interfacing with their molecular structure, and providing a liquid system for reactivity making it an essential of life. We live on a watery planet and aquaporins reflect this condition in cells and their adaptations. Aquaporins, not only channel water but many fundamental compounds of life. They are widespread in the human body where their evolution can be studied throughout the tree of life, making them the best candidate to model both micro and macroevolutionary change.

In the geological time scale, through specific cellular adaptations, water and aquaporins can tie together the broad and molecular concepts of biology. Through the aquaporin lens, students can connect the big picture of a water planet over billions of years to the manifestation and an adaptation in a cell membrane to the hydrological cycle. Through a genomic medicine lens students can witness mutations that alter the function of the aquaporin protein and the changes that may produce a disease phenotype, and through phylogenetic trees they can see everything from desiccation strategies to diseases. AQPs reframe our relationship with our planet over its 4.5-billion-year history and the water-based life forms that emerge from it.

○ Supplemental Material

Supplementary teaching material is available on the genomic medicine website at Temple University. Please visit our website www.GenomicMedicine@temple.edu for teaching material and images and activities.

We have 4 activities:

1. Timetree of life: Desert vs non desert animals and aquaporins
2. Color in aquaporins of the human body: distribution and adaptation
3. What's your aquaporin hypothesis?
4. MEGA: Building an aquaporin tree

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