



## Review

## Aquaporins: Another piece in the osmotic puzzle

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## ABSTRACT

**Osmolarity not only plays a key role in cellular homeostasis but also challenges cell survival. The molecular understanding of osmosis has not yet been completely achieved, and the discovery of aquaporins as molecular entities involved in water transport has caused osmosis to again become a focus of research. The main questions that need to be answered are the mechanism underlying the osmotic permeability coefficients and the extent to which aquaporins change our understanding of osmosis. Here, attempts to answer these questions are discussed. Critical aspects of the state of the state of knowledge on osmosis, a topic that has been studied since 19th century, are reviewed and integrated with the available information provided by in vivo, in vitro and in silico approaches. © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.**

### 1. Introduction

Among the environmental factors that play significant roles in cellular homeostasis, pH and osmolarity are both essential, as demonstrated by the multiple cellular responses they can trigger to effect the appropriate cellular adjustments. However, while pH control is understood at the molecular level, the mechanisms underlying osmosis remain unknown.

Osmosis is not restricted to living organisms, though it is especially relevant for the life sciences because it involves the movement of water. A total of 60–95% of the weight of active living cells is due to water. Moreover, as a solvent, water determines many aspects of the function of molecules, cells and organisms; so studies about osmosis are central to understanding how water moves in and out of cells, as well as between cell compartments. The elucidation of osmotic phenomena will help to understand central issues such as the identification of the causes of previously identified syndromes (e.g., neuromyelitis optica) and could also aid in finding adequate therapies for various pathologies, the comprehension of water management by plants, and the development of efficient methods for water purification. Therefore, unveiling the osmotic process is important both at the biological and technological level [1].

Osmosis is a long-known phenomenon that has been under investigation since the beginning of 19th century. Two major milestones in the history of its scientific study include: (i) Dutrochet's preliminary evidence of the existence of an osmotic pressure gradient and the development of the first known osmometric device [2] and (ii) the plant physiologist Pfeffer's first measurements of osmotic pressures using artificial semipermeable membranes [3]. These events were of great impact on the future theories proposed to explain osmosis because they provided not only a way to measure osmotic pressure but also a relationship between this pressure and solute concentration. However, despite the solid foundation that these contributions made at a phenomenological level, it was not until 1887 that the first physical theory for osmotic equilibrium was stated in van't Hoff memorable paper entitled *The Function of Osmotic Pressure in the Analogy between Solutions and Gases* [4].

van't Hoff's osmotic theory was based on the description of gases and liquid solutions involved in an osmotic equilibrium through a semipermeable membrane. This key point of the theory is not a minor aspect because this description, which is discussed and questioned later in this review, opened the door for the mathematical treatment of osmosis and its inclusion into the mainstream of science.

Later, in 1901, van't Hoff was honored with the Nobel Prize in Chemistry, and during his Nobel Lecture, he remarked on the relevance of this phenomenon at the biological level.

In van't Hoff words [112]:

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Whereas application of the laws of osmosis has proved very fruitful in the field of chemistry, what De Vries and Donders emphasized 15 years ago, namely that osmotic pressure plays a fundamental role in plant and animal life, has since been fully confirmed as well. The determination of osmotic pressure and of the associated lowering of the freezing-point of solutions is already frequently of great importance in physiology and medicine, e.g., in the study of disease. However, the peculiar discovery made very recently by Loeb is the most important one of all. This scientist has been studying the problem of fertilization, which is bound up so closely with the problem of life, and he has found that the eggs of sea urchins will develop as a result of the temporary action of a specific osmotic pressure brought about by solutions of potassium chloride, magnesium chloride, sugar, etc.

In the era of molecular biology, studies of osmosis have been somewhat neglected in science. However, in the last decade, the discovery of aquaporins returned osmosis to the center of the research scene. As Portella & de Groot explain, “Water permeation through channels of molecular dimensions is therefore of great interest in biology, but also in technological applications: water-selective pores, such as aquaporins, are suitable as filtering devices.” [5]. Under this scenario, we would like to review some critical aspects that might help clarify the state of the field of osmosis.

## 2. Diachronic analysis of the study of osmosis

As previously mentioned, the first theory about osmosis was presented in 1887 by van't Hoff [4]. The main law of this theory is still used today and is usually presented as follows:

$$\Pi = RTC \quad (1)$$

where  $\Pi$  is the osmotic pressure,  $R$  is the gas constant,  $T$  is the temperature and  $C$  is the solute concentration. This equation was originally based on the idea that with sufficiently dilute solutions the osmotic pressure will be the same as the dissolved substance would exert as gas. After Gibbs contribution to thermodynamics it became clear that the osmotic pressure is not the result of the solute molecules impact against a semi-permeable membrane but the pressure necessary to compensate the free energy deficit owing to the solute dissolution into the solvent [6].

During the 1950s, a number of physiologists studied water movements across epithelial membranes involving osmotic water fluxes coupled to solute transport [7,8]. However, it was not until the contributions of Kedem–Katchalsky [9–11], which were based on the previous contributions of irreversible thermodynamics [12–14], that the osmotic process was understood in terms of both solvent and solute fluxes across membranes on the basis of thermodynamics. The equations describing solvent flow through semipermeable membranes proposed by Kedem–Katchalsky can be written as follows:

$$J_v = L_p \Delta P - L_p \sigma \Delta \Pi \quad (2)$$

$$j_s = \omega L_p \Delta \Pi + (1 - \sigma) \bar{c} J_v \quad (3)$$

where  $J_v$  is the volume flux,  $j_s$  is the solute flux,  $\Delta P$  and  $\Delta \Pi$  are the pressure and osmotic differences on both sides of the semipermeable membrane, respectively,  $L_p$ ,  $\omega$  and  $\sigma$  are transport coefficients, and finally,  $\bar{c}$  is the mean solute concentration. This formalism derived from irreversible thermodynamics has governed the studies of osmosis until now. Although this approach has been demonstrated to be robust enough to allow the description of biological systems, it has also been the focus of controversy [6,15,16]. The formalism of the Kedem–Katchalsky proposal was generally accepted, though considerations of “the fundamental cause of osmosis” [17] were still open to discussion. Although it is beyond the scope of this

article to analyze the meaning of “the fundamental cause of osmosis” from an epistemological point of view, we would like to draw attention to the continuing search for another theoretical framework that explains the molecular mechanisms of the osmotic phenomenon.

In the late 1970s, physiologists deeply discussed the nature of the osmotic process in the famous *Forum in Osmosis* organized in *Am. J. Physiol.* [17–21]. The core of those discussions was based on mechanical statements made prior to thermodynamics. Finally, in 1987, Finkelstein wrote an important systematization of both the theoretical and experimental features of water osmotic fluxes [6]. Interestingly, the main progress made during those years was not in terms of the elucidation of osmosis itself but in introducing an important concept in biological systems: the prediction of the existence of protein entities with the ability to transport water through biological membranes, that is, an alternative to the lipid pathway.

### 2.1. Hydraulic and permeability coefficients

The typical experimental approach to reveal an osmotic phenomenon is to separate two solutions of different solute concentration (for at least one solute) by a membrane that allows water movements and obstructs solute transport across it. In such a system, we can distinguish some observable features, such as the solute concentration difference between both solutions ( $\Delta \Pi$ ) and the volume flux ( $J_v$ ) from the diluted compartment to the more concentrated one. These observables have been combined, as mentioned above, in a thermodynamic framework by Kedem–Katchalsky [9], and the quotient between them, i.e., the phenomenological coefficient ( $L_p$ ) became relevant.

$L_p$  is known as the *hydraulic permeability coefficient* and, strictly speaking, it relates  $J_v$  and  $(\Delta P - \sigma \Delta \Pi)$ . In particular,  $L_p$  relates the flux developed with the osmotically driven force, and becomes a useful tool itself for characterizing the membrane properties in terms of its semi-permeability only if the following hold true: (i) the volume flux is water flux, i.e., there is no solute transport through the membrane, and (ii) the driving force is osmosis, i.e., there is an absence of hydrostatic pressure differences.

Based on the contributions of Kedem–Katchalsky, a plethora of alternative coefficients were proposed according to the units employed. Of course, these coefficients are not essentially different from one another, and given such equivalence among them, this review will focus on osmotic permeability ( $P_f$ ). The relationship between  $L_p$  and  $P_f$  is:

$$P_f = \frac{L_p RT}{AV_w} \quad (4)$$

where  $V_w$  is the water partial molar volume,  $R$  is the gas constant,  $T$  is the temperature and  $A$  is the area of the membrane. This expression is generally used when the volume flux is considered in water mole  $s^{-1}$  instead of  $cm^3 s^{-1}$ . In these conditions, and considering only the situation in which the solutes do not cross the membrane at all, Eq. (2) is rewritten as:

$$\phi_w = P_f A \left( \frac{\Delta P}{RT} - \Delta c_s \right) \quad (5)$$

where  $\phi_w$  is the volume flux in mole  $s^{-1}$  and  $\Delta c_s$  is the solute concentration difference between the compartments separated by the membrane.

It is important to stress that these coefficients are crucial for the comprehension of the phenomenon; they vary depending on the membrane structure and the nature of the solutes that cannot cross it [22]. Thus, for a given  $\Delta \Pi$ , the magnitude of the volume flux can be distinct for different systems, and those differences will

give clues not only about the membrane structure but also about the molecular events involved in the transport through the membrane. As mentioned before, inquiring about such coefficients was relevant and essential for later predictions of the presence of pores in the membrane.

Water transport through biological membranes has long been the focus of scientific interest both for the importance of the transport itself and for the contributions these studies could make to membrane structure elucidation [23]. Indeed, in the 1950s, Stein and Danielli proposed the existence of hydrophilic pores responsible for water and ion movements through membranes [24]. Notwithstanding, only the proper measurement of water transport coefficients provided strong support for the hypothesis that water moves not only across lipids by partition-diffusion events but also, and predominantly, through pores [6].

Continuing our analysis of the most relevant coefficients, if a driving force is established at both sides of a semipermeable membrane, there will be a volume flux. The driving force could be hydrostatic pressure, osmotic pressure or both. Coefficients such as  $P_f$  can be obtained by conducting an experiment in which a driving force is established on both sides of a semipermeable membrane and the consequent fluxes are measured. On the other hand, in the absence of a driving force such as that produced by osmotic or hydraulic pressure, water is also expected to move, in this case by diffusion. The coefficient that represents the diffusion of water through a membrane can be obtained by measuring the flux of tritiated water molecules that cross the membrane when a non-tritiated water concentration gradient is established on both sides of the membrane. This coefficient has historically been named  $P_d$  (water diffusion permeability coefficient).

Finkelstein elegantly discussed the biophysical arguments that allow a theoretical speculation on the mechanisms of osmosis, stating that the ratio  $P_f/P_d$  should be 1 ( $P_f/P_d = 1$ ) if water moves by a partition-diffusion process during osmotic events [6,25]. Thus, a ratio *different* from 1 should indicate that water could be moving through pores. In this context, the measurement of the transport coefficients  $P_f$  and  $P_d$  was useful for understanding the nature of the path for water transport in biological membranes.

## 2.2. Aquaporins on the stage

It is historically relevant to consider the confluence of three research approaches involved in the discovery of aquaporins. We can describe them as follows: (i) the biophysical, (ii) the bioinformatics and (iii) the serendipity approaches. We will summarize briefly how these research approaches contributed to the discovery of water channels.

### 2.2.1. The biophysical route

Peter Agre won the Nobel Prize for the discovery of aquaporins and introduced his Nobel Lecture with these words [113]:

I wish to discuss the background in order to give credit to the individuals who were in this field long before we joined the field. With the recognition of the lipid bilayer as the plasma membranes of cells back in the 1920's, it was correctly proposed that water could move through the membrane simply by diffusing through the lipid bilayer. The current view is that the lipid bilayer has a finite permeability for water, but, in addition, a set of proteins exists that we now refer to as "aquaporins". Their existence was suggested by a group of pioneers in the water transport field who preceded us by decades – people including Arthur K. Solomon in Boston, Alan Finkelstein in New York, Robert Macey in Berkeley, Gheorghe Benga in Romania, Guillermo Whitttembury in Venezuela, Mario Parisi in Argentina – who by biophysical methods predicted that water channels

must exist in certain cell types with high water permeability such as renal tubules, salivary glands, and red cells (reviewed by Finkelstein, 1987 [6]).

This citation illustrates what we define here as the biophysical route. Early in the 1960s, it was known that some biological membranes exhibit high water permeability [26,27]. Frog skin [28] and frog urinary bladders [29] were tested. However, red blood cells have always been the preferred system to study water transport, not only due to their availability but also because red blood cells have only one membrane that can limit water movements. Solomon and coworkers found that  $P_f$  was 2.5 times greater than  $P_d$  in red blood cells [30,31]. This result was interpreted, under the theoretical approximation used in the experimental design, as indicating the existence of a pore that can mediate the osmotic movement of water in the membranes of those cells. Moreover, some years later, Macey and Farmer reported another result suggesting the existence of protein pores. They found that sulfhydryl reagents dramatically reduced red blood cell osmotic water permeability [32]. Those years were of intense and challenging scientific production. Predictions about the existence of water pores led to increased research, and important advances were achieved. Benga and coworkers made significant progress in identifying a protein which could be the hypothetical red blood cell pore [33,34]. Parisi and coworkers detected that pH was a putative inhibitor of the water pathway [35] (it was later demonstrated that the water channels are closed by acidification [36,37]). Moreover, the presence of the water pore among other proteins was probed [38]. In the mid-1980s, sufficient evidence of the existence of water channels in biological membranes of different animal tissues was recognized (for a review see [39]).

The only pending discovery was the isolation of the water pore that possessed the water transport activity. That final thrust was made thanks to the other research routes that converged at this point in the story.

### 2.2.2. The bioinformatic route

In addition to the biophysical achievements and during the late 1980s, a growing family of homologous membrane proteins was recognized. Several proteins from quite diverse organisms shared high sequence identity, but their functions were not clear [40]. Those proteins were as follows: (i) the major intrinsic protein (MIP) from bovine lens fiber junction membranes [41], (ii) the plant nodulin-26 (NOD) from the peribacteroid membrane root nodules [42], (iii) the glycerol facilitator (GLP) from *Escherichia coli* [43], (iv) a tonoplast intrinsic protein (TIP) of higher plant seeds [44], (v) a protein termed Big Brain (BIB) from *Drosophila* [45] and (vi) a putative glycerol facilitator of *Streptomyces coelicolor* (GLY) [46]. All of these proteins had a similar size, ranging from 256 to 281 amino acids, and contained six putative membrane-spanning domains. Most importantly, they all seemed to share a common ancestor. The different localization and sources of these proteins caused confusion about their function because similar functionality was expected for similar sequences and the relationship among these proteins was not clear in this regard [47].

In 1991, Agre and co-workers published the isolation of a cDNA named CHIP28 from different mammalian tissues, and its amino acid sequence revealed a strong homology with the major intrinsic protein of bovine lens. CHIP28 became categorized as a member of the MIP family [48]. Advances made in the identification of MIP family members allowed scientists to hypothesize about their role as water movement channels [40,48].

### 2.2.3. The serendipity route

In 2005, two years after winning the Nobel Prize for his discovery of aquaporins, Peter Agre held a conference at the American Thoracic Society [49] stating the following:

Our laboratory got into the water channel field by accident. We discovered a polypeptide in red cells that we didn't expect to see.

Later, in an interview with a journalist from The New York Times [50], Dr. Agre explained in detail how he and co-workers discovered that CHIP28 was a water channel:

By serendipity. We had an NIH grant to study the Rh blood group antigen. We had developed a method to isolate the Rh molecule. And a second protein called 28K kept appearing in the tests. At first, we thought 28K was a piece of the Rh molecule, some kind of breakdown product of the Rh, a contaminant that showed during testing. But as we studied it further, 28K seemed to be an undiscovered molecule. No scientist had ever reported it before. But what did it do?

As a part-time project on weekends, we pursued that question. We calculated 28K presence in different types of cells. This mysterious protein was enormously abundant in red blood cells and kidney tubules. And after we cloned and sequenced it, we found it to be related to a series of proteins of very diverse origins, like the brains of fruit flies, bacteria, the lenses of eyes, even plant tissues. Still: what was it? Then in 1991, I visited John Parker (He died in 1993). He'd been my hematology professor at the University of North Carolina. He said, "boy, this thing is found in red cells, kidney tubules, plant tissues; have you considered it might be the long-sought water channel?" It was his suggestion that caused me to change the direction of my research. What my lab team was able subsequently to prove was that 28K formed these little tubes inside many cells and that water passed through them. With that, more than 100 years of scientific controversy was ended.

Peter Agre was a hematologist who was not searching for the molecular entity responsible for water transport in biological membranes. However, he and his co-workers were clever enough to detect that what they first found and considered to be an obstacle, turned out to be unexpectedly important for science. Agre's group cloned the cDNA of the 28 kDa protein and obtained a 269-amino acid polypeptide, which was named CHIP28 [50]. The analysis of the CHIP28 primary sequence revealed a strong identity with the major intrinsic protein of the bovine lens, indicating that CHIP28 could be part of the MIP family. However, the crucial experiment was the measurement of CHIP28 water transport activity. Agre and co-workers assayed osmotic CHIP28 water permeability by expressing its cRNA in *Xenopus laevis* oocytes and submitting those oocytes to an osmotic shock. Injection of CHIP28 mRNA into *Xenopus* oocytes resulted in a dramatic swelling of the oocytes followed by their rupture within 5 min, whereas control oocytes failed to swell even after incubations of more than one hour [51,52]. Undoubtedly, CHIP28 was the much sought after water pore.

### 3. What do we know about aquaporins today?

#### 3.1. Structural and functional characteristics of water channels

The aquaporin family is present in all kingdoms of life; aquaporins have been described in bacteria, yeast, invertebrates, vertebrates and plants. However, the mammalian and vascular plant water channels are the most studied. Interestingly, while there are 13 different aquaporins in mammals, from AQP0 to AQP12 [53], more than 30 water channels have been found in plant genomes. These aquaporins are classically classified into seven subfamilies: PIPs (plasma membrane intrinsic proteins), TIPs (tonoplast intrinsic proteins), NIPs (NOD26-like intrinsic proteins), SIPs (small basic intrinsic proteins), XIPs (x intrinsic proteins), HIPs

(hybrid intrinsic proteins) and GIPs (GlpF-like intrinsic proteins) [54–56]. The presence of aquaporins in all living organisms suggests the importance of fluid homeostasis and a related positive selection pressure for proteins associated with this process [57]. Notwithstanding, a full understanding of the mechanisms underlying water channel diversity has not yet been achieved.

From 1992 to today, more than 6000 articles have been indexed under the tag "aquaporin" in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), giving a publication rate of almost one scientific paper per day since the discovery of AQP1. This tremendous expansion in this type of research has enabled a detailed picture of what a water channel is. The first astonishing finding may be that aquaporins are not specific water channels because most members of this huge family are also able to transport other molecules such as glycerol, urea and arsenic, among others. However, it seems that the main characteristic of this family is that it is part of an evolutionary tree that facilitated water transport as an ancestral feature [58].

At least two levels of information about water channel structure are the key to understanding how water crosses aquaporins: (i) the molecular water path itself, as an enabler of water molecule movement once it enters the channel, and (ii) the protein conformational changes that control the opening and closing of that path.

Regarding the path itself, a detailed description of the molecular aspects of water and solute transport through aquaporins has been elucidated in structural studies of GlpF and AQP1 [59–61]. Two pore constrictions that act as selectivity filters have been identified: the NPA region and the so-called Ar/R region located on the extra-cytoplasmic entrance of the pore [55,62,63]. The complementation between the structural approaches and simulation strategies revealed that water molecule dipoles inside the channel were oriented within the NPA region, thus allowing proton exclusion from the interior of the channel by a large electrostatic barrier that peaks at the NPA site. On the other hand, the Ar/R region acts as the selectivity filter for uncharged solutes [64].

With respect to protein structure, all aquaporins seem to share the following common features: they (i) are transmembrane proteins, (ii) have six transmembrane domains, with the N- and C-termini localized in the cytosol, (iii) have two duplicated halves, each of which include the NPA site that acts as a selectivity filter, and (iv) are organized as tetramers in which each monomer is a functional unit.

The unveiling of relevant structure-function relationships in aquaporins was the result of the confluence of techniques and theory. Even before crystallographic studies, the water permeation in water channels was suggested as a single-file process, i.e., water molecules form a special structure inside the channel where one molecule travels through after another with no gaps between them. Moreover, all of the water molecules move in a concerted fashion [6]. Despite some experimental evidence that was interpreted to corroborate the single file transport mechanism, this mechanism was considered an oversimplification by de Groot and coworkers because water molecules might occasionally interchange positions [64,65].

The interplay between aquaporins and water started to be elucidated in the last decade through molecular dynamics simulations [5]. Thanks to this methodology, it became clear that the confinement of water in the vicinity of molecular interfaces could change its structural and dynamic properties. For instance, it was recently demonstrated that the hydrogen bond distribution, a structural property of water, is strongly influenced by a hydrophobic surface even in a distance greater than the thickness of the plasma membrane (80 Å) [66]. However, we still do not have a complete understanding of water transport events when continuous models for fluids cannot be applied. Portella and de Groot noted that the modifications of the macroscopic hydrodynamic equations that were

made to account for the diffusive nature of the permeability coefficients were empirical and ad hoc, rather than based on solid physical theories [5], so care must be taken when extrapolating results.

The other main aspect of aquaporin molecules is that they are, similar to other channels, i.e. rigorously regulated. It is well established that eukaryotic aquaporins are often directly regulated by pH [36,37,67–70], phosphorylation [71–74] and divalent cations [37,68,75–77]. Protonation, phosphorylation and the binding of cations directly affect protein conformation, which, in turn, impacts its transport activity. Moreover, for some aquaporins, the mechanism of this conformational transition from a closed state to an open one has been elucidated [78].

This elucidation has been achieved in the case of the plant aquaporins known as PIPs. Their closed conformation involves the movement of loop D to a position that blocks the water pore after the protonation or phosphorylation of conserved residues. However, unlike the profound knowledge gained in unraveling pH gating in plant PIPs, the mechanisms of gating for the other water channels are not yet clear. For example, the gating of AQP0 seems to involve conformational changes in loop A, whereas for bacterial AqpZ, changes in the conformation of the Ar/R filter may be responsible for the opening and closing of the channel [78].

Aquaporin regulation not only includes the gating of its channels but also the coordinated targeting of the proteins to different membranes. AQP2 is an example of this strict regulatory mechanism. The AQP2 channel exhibits a translocation from the intracellular space to the apical membrane of the collecting duct cells after the hormone ADH binds to the V2 receptor. The events that are described to occur as a consequence of ADH binding to its receptor involve the phosphorylation of AQP2 and the subsequent translocation of the vesicles that contain this channel at the apical membrane (for a review see [79]). Moreover, the translocation of AQP1 under a hypotonic stimulus was recently reported, thus allowing the cell to control the number of water channels in its membrane [80]. Among the plant aquaporins, trafficking is also a critical point for water transport regulation. This mechanism is being studied mainly in the PIPs (reviewed in [81]). Some PIP1 isoforms fail to be functionally expressed in the plasma membranes of *Xenopus oocytes*. In addition, Fetter and coworkers provided experimental data supporting the conclusion that this event could be related to a specific interaction between PIP1 and PIP2 proteins because the co-expression of PIP 1s and PIP2s in *Xenopus oocytes* enables PIP1 insertion in the plasma membrane [82,83]. As recent reports have suggested, it is likely that the constitutive cycling of PIPs is an essential component of their function and regulation under both resting and stress conditions [84].

### 3.2. Participation of aquaporins in physiological processes

Currently, water management is being studied on the organismal and cellular levels. In addition, subcellular studies are also being conducted in order to understand water transport pathways.

As explained by Eric Beitz in the prologue to *Aquaporins* (2009) [85], failures in the performance of mammalian aquaporins are usually associated with diseases such as nephrogenic diabetes insipidus and Sjogren's syndrome, and moreover, aquaporin water and solute permeability have also been implicated in processes such as lung and brain edema, obesity, tumor angiogenesis and wound healing [85]. For instance, the elucidation of the molecular events related to the regulatory volume decrease mechanism of mammalian cells [86] and the water transport properties of vital organs such as the liver [87,88] are being investigated to determine the participation of aquaporins.

Despite the fact that there is a vast and specific literature that has deepened the knowledge of mammalian AQPs, we would like

to mention two examples of scientific achievements in which *in vivo* experimentation was crucial to further the understanding of pathophysiological situations. Specifically, mouse models have been used to study the role of AQP1 and AQP4 in mammals.

In the case of AQP1, it was observed that mice lacking AQP1 demonstrated reduced tumor growth after the injection of melanoma cells [89]. Interestingly, experiments designed to elucidate the mechanism of defective tumor angiogenesis also revealed that cultured aortic endothelial cells from AQP1-null mice presented with a slow rate of migration. The participation of AQP1 in cell migration was then proposed, and a model for these events was developed. As a consequence of actin polymerization–depolymerization and transmembrane ionic fluxes, the cytoplasm adjacent to the leading edge of the migrating cells undergoes rapid changes in osmolality, and AQPs facilitate the rapid cell volume changes that enable cell propulsion. Of course, in the absence of AQPs, there is also cell migration because water can cross the cell membrane through the lipid bilayer; however, this process will occur much more slowly than water transport through AQPs [90].

In the case of AQP4, a glial membrane water channel, the phenotypic analysis of AQP4-deficient mice opened up new possibilities regarding the mechanisms of water transport during the development of cerebral edema, indicating a putative role for AQP4 in cellular water uptake into the brain as well as the clearance of extracellular fluid from the brain [91]. In this work, the authors showed that mice deficient in AQP4 have much better survival than wild-type mice in a model of brain edema caused by acute water intoxication. This result suggests a role for AQP4 in the modulation of water transport in the brain. This finding resulted in the proposal of AQP4 inhibition as a new therapeutic option not only in brain edema but also in a wide variety of cerebral disorders.

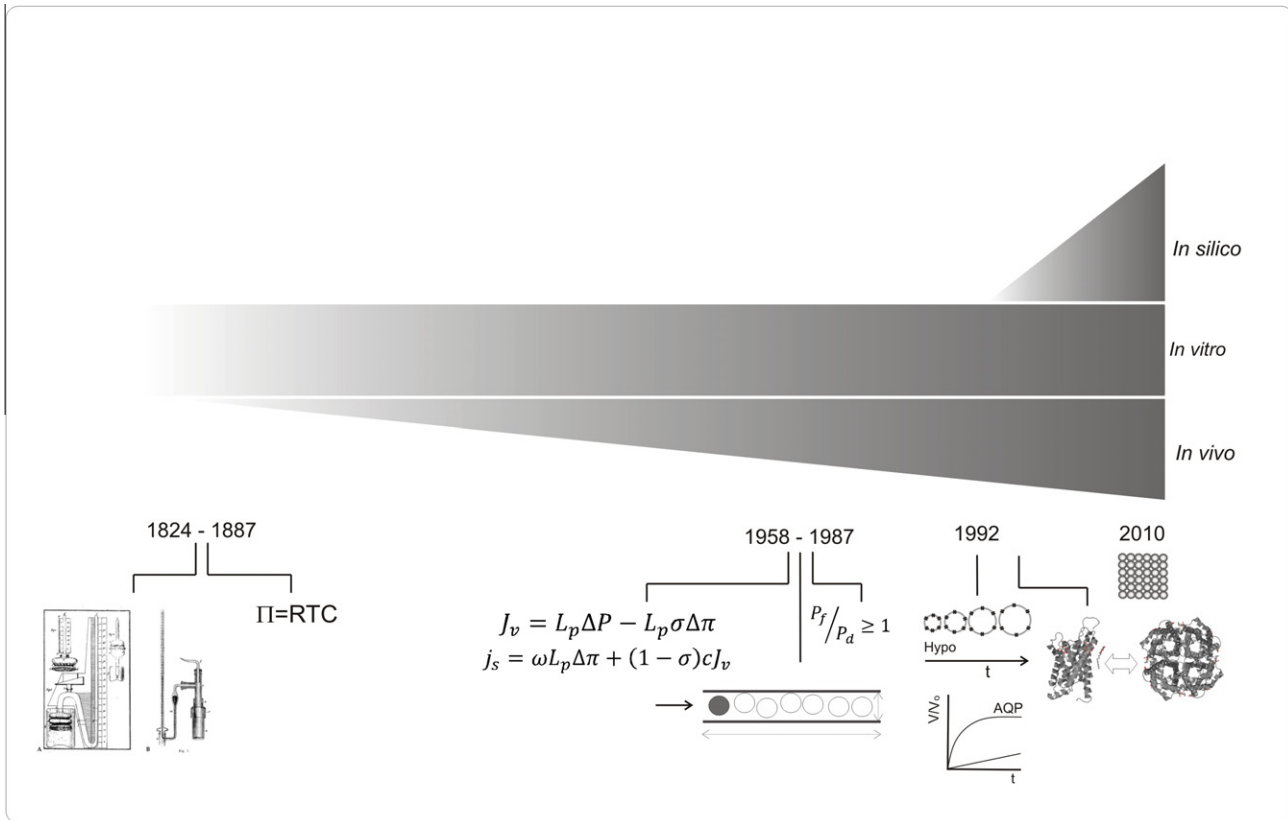
Concerning research in the plant biology field, Christophe Maurel [92] states that:

...studies on the integrated function of aquaporins in plants have been somehow limited by the high genetic diversity of aquaporins in these organisms, the lack of specific inhibitors, and methodological difficulties for measuring water transport.

Despite the evident complexity of the field, there is currently a significant amount of evidence that conclusively shows that plant aquaporins do indeed contribute to water (and other solutes) transport in roots [93–95], leaf and petal movements [74,93,96], seed germination [97], pollen [98] and fruit ripening [69,99], among other processes. An example of aquaporin participation in key physiological processes in plants is the investigation related to aquaporin-mediated changes in hydraulic conductivity ( $L_{pr}$ ) in whole maize plants [100]. In this previous study, when  $L_{pr}$  was manipulated using different treatments that inhibit aquaporins, it was found that cell turgor measured in the elongating zone of leaves decreased synchronously with  $L_{pr}$  and that the leaf elongation rate followed those changes. These results could be interpreted as indicating that stimulus-induced changes in root water transport induce a reduction in leaf cell water potential (turgor), which results in arrested cell growth [101].

What is interesting about these observations in the field of plants is that reports indicate that aquaporins are unexpected players in key physiological processes. In the case of water uptake by roots, the radial transport path (i.e., between root epidermis and xylem) is considered the main hydraulic barrier, and studies similar to that described above are aimed at gauging the role of the transcellular component.

A second example of the role of aquaporins in physiological processes could be solute transport. Ion channels and transporters are considered to play key roles in plant nutrient absorption. The



**Fig. 1.** A schematic diagram showing the main historical landmarks since osmosis was addressed for the first time. The discovery of aquaporins, as well as the previous biophysical framework, are indicated. Bars represent the relative contributions to the field supported by different approaches (in vitro, in vivo and in silico). The discovery of aquaporins coincided with a scientific period enriched in terms of new methodologies, techniques and tools (including genomics and bioinformatics) that might reflect the increase in research in this topic from their initial discovery until the present day.

estimated number of particles (ions or solutes) translocated per second differ for carriers (200–50000) and ion channels ( $10^6$ – $10^8$ , open state), and considering the diversity of both entities, this window with two ranges was considered sufficient to explain nutrient uptake. However, examples in the literature have confirmed an unexpected role of aquaporins in solute absorption. For instance, it was demonstrated that the aquaporin NIP5;1 is a major plasma membrane boric acid channel crucial for the B uptake required for plant growth and development under B limitation [102]. NIP5;1 facilitates the transport of boric acid in addition to water, and in *Arabidopsis thaliana*, the gene is strongly upregulated in the root elongation zone and in the root hair zone under B limitation. Compared to ion channels and transporters, aquaporins are  $10^9$  times faster, and the presence of solute permeable aquaporins represents a new concept in terms of absorption, not only because of the rate of the process but also in its osmotic implications.

#### 4. Aquaporins in the framework of phenomenological and mechanistic osmotic theories

The importance of water for life is accepted worldwide. Notwithstanding, scientists studying the movement of this solvent into and out of cells have faced a question that at a first glance appears simple, but is actually quite difficult; that is, how does water cross biological membranes?

When the lipid bilayer was discovered in the 1920s, it was speculated that water permeability would take place by diffusion through lipids. However, as explained in previous sections, the incompatibility between the measured permeability of mammal tissues and the theoretical rates of diffusion of water in lipids

suggested other pathways for water, mainly protein channels. The biophysical differences between both types of pathways for water are major. Whereas diffusion is a low capacity movement of water, channels are high capacity, highly selective and can be controlled by regulatory mechanisms.

As Giuseppe Calamita explains in the Editorial for the *Biology of the Cell Aquaporin Collection* [103],

...although simple diffusion of water across the lipid bilayer occurs through all biological membranes, its low velocity and finite extent soon became apparent, suggesting the existence of additional pathways for water moving through the membrane. In spite of the enormous amount of work carried out in this area, the precise and complete answer only came relatively recent with the discovery of the aquaporins, making the membrane 10- to 100-fold more permeable to water than membranes lacking such channels.

In this paragraph, the actual state of the puzzle is summarized. A given osmotic gradient across a membrane induces an osmotic flow. The osmotic flow can be achieved through the lipid bilayer as well as through proteins. It is important to remark that aquaporins are the main water transporters, but it must also be considered that other proteinaceous channels have been proposed to enable water to cross the lipid bilayer [104,105]. Both pathways differ in terms of molecular identity, permeability, selectivity, and efficiency. In fact, the up-regulation of the water transport capacity of a membrane with aquaporins can improve the osmotic flux generated by the osmotic gradient. As previously mentioned, the hormone ADH increases cell membrane osmotic permeability in kidney cells due to the incorporation of AQP2 into the membrane

[106]. On the other hand, the activation energy of the transport through the membrane can be reduced by the incorporation of water channels, i.e., the energy that water molecules must consume to cross the membrane is lower when aquaporins are present among the lipids.

In this context, the following question is mandatory: How are aquaporins incorporated within the phenomenological point of view in the study of osmosis? The answer is simple but not trivial. That is, the performance of aquaporins will be encrypted on the magnitude that the phenomenological coefficients are acquired. In the framework of this theory, a membrane undergoing aquaporin up-regulation will show a numerical increase in the parameter  $P_f$ . Therefore, the strength of the phenomenological theory of osmosis is clear in that a single parameter can deal with changes in the biochemical complexity found among a lipid bilayer and a protein channel. However, along with its robustness, the predictive capability of the theory is limited. A phenomenological theory cannot offer mechanical details regarding the development of the phenomenon. Mechanistic theories are suitable for extending the frontiers of the theoretical predictions.

The tension between the unquestionable usefulness of irreversible thermodynamics and the requirement for a mechanical characterization of osmosis can be found in many publications [8,107–109]. As noted by Hill and coworkers [110,111], it is not possible to use phenomenology if the purpose is to unveil mechanistic events once it was revealed that aquaporins are true players in osmotic water transport. As mentioned above, the robustness of phenomenological coefficients is their own limitation. Osmotic coefficients, such as  $P_f$ , can measure the water permeability of a membrane *as a whole*, but cannot separately describe the two main pathways for this transport. If we consider, following Curry and coworkers [110,111], that the membrane is a *mosaic* composed of at least two elements for water transport, Kedem–Katchalsky equations must be used carefully to prevent the misinterpretation of experimental data. A deeper understanding of a composite model of water pathways and the ways they interplay within the theories underlying osmosis are still needed.

## 5. Concluding remarks

Many scientists have mentioned the importance of finding a mechanistic explanation for the osmotic phenomenon, and much of the complexity of the history of osmosis is due to the constant search for a mechanism that can explain the event at a molecular level. Proteinaceous pores involved in water movement in biological systems have been found, and this finding was supposed to answer unsolved questions. However, even with progress in the clarification of concepts made by mechanistic analyses, most experimental approaches to study osmotic transport in cells are still based on the measurements of the hydraulic characteristics of the water pathways by means of macroscopic transport coefficients.

At this crucial point, two main theoretical approaches coexist: the irreversible thermodynamics [9] and some mechanistic reinterpretations of it (i.e., the proposal of [111]). Moreover, a lot of information is available regarding water channel structures together with significant knowledge about water management by cells under different physiological and pathophysiological conditions (Fig. 1). Hence, it seems evident that while the discovery of aquaporins did not radically change our conceptualization of osmosis, the rationale of aquaporins can still be addressed from other perspectives. Hill has defined the “*simple permeability hypothesis*” as the idea that gives aquaporins the crucial role of modulating the osmotic permeability of membranes, assuming that without them, a living organism “would not be able to sustain net water movement at a rate suitable for fulfilling certain cellular

or transcellular functions” [111]. Without discarding the widely proven fact that aquaporins indeed increase membrane permeabilities, considerable experimental evidence that has been well summarized in Hill’s work rules out this naïve fate for water channels. Alternatively, Hill and coworkers proposed interesting possibilities for water channels, for instance, that they are osmotic or turgor sensors. These interesting possibilities for AQPs make the osmotic phenomenon even more complex, but still place water channels in the preexistent theoretical framework. Therefore, the concepts have not changed thus far; however, the evidence obtained with systems expressing AQPs that were subjected to osmosis, coming from *in vivo*, *in vitro* or *in silico* experiments, constitute a challenge for scientists if the regulatory aspects of osmosis in biological systems are intended be part of the whole puzzle. Links between all of these contributions needs to be deepened to follow the path outlined by van’t Hoff a hundred years ago, i.e., to clarify how osmosis is involved in the development and maintenance of life. The following years will demonstrate to what extent this challenge is addressed.

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## References

- [1] Nielsen, C.H. (2010) Major intrinsic proteins in biomimetic membranes. *Adv. Exp. Med. Biol.* 679, 127–142.
- [2] Dutrochet, H. (1828) Nouvelles recherches sur l’endosmose et l’exosmose: suivies de l’application expérimentale de ces actions physiques à la solution du problème de l’irritabilité végétale et à la détermination de la cause de l’ascension des tiges et de la descente des racines. Paris.
- [3] Pfeffer W. (1877) Osmotische Untersuchungen. Studien zur Zell mechanik. Wilhelm Engelmann Leipzig.
- [4] van’t Hoff, J.H. (1887) Die Rolle des OsmotischenDruckes in der AnalogiezwischenLosungen und Gasen. *Z. Phys. Chem.* 1, 481–493.
- [5] Portella, G. and de Groot, B.L. (2009) Determinants of water permeability through nanoscopic hydrophilic channels. *Biophys. J.* 96, 925–938.
- [6] Finkelstein, A. (1987) Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes: Theory and Reality, John Wiley & Sons Ltd., New York.
- [7] Ussing, H.H. (1952) Some aspects of the application of tracers impermeability studies. *Adv. Enzymol. Relat. Subj. Biochem.* 13, 21–65.
- [8] Pappenheimer, J.R. (1953) Passage of molecules through capillary walls. *Physiol. Revs.* 33, 387.
- [9] Kedem, O. and Katchalsky, A. (1958) Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochem. Biophys. Acta* 27, 229–246.
- [10] Kedem, O. and Katchalsky, A. (1961) A physical interpretation of the phenomenological coefficients of membrane permeability. *J. Gen. Physiol.* 45, 143–179.
- [11] Katchalsky, A. and Curran, P.F. (1965) Non-equilibrium Thermodynamics in Biophysics, Harvard University Press, Cambridge, MA, USA, pp. 113–132.
- [12] Staverman, A.J. (1951) The theory of measurement of osmotic pressure. *Rec. Trav. Chim.* 70, 344–352.
- [13] Staverman, A.J. (1952) Non-equilibrium thermodynamics of membrane. *Trans. Faraday Soc.* 48, 176–185.
- [14] Kirkwood, J.G. (1954) Transport of ions through biological membranes from the standpoint of irreversible thermodynamics in: *Ion Transport Across Membranes* (Clark, H.T., Ed.), pp. 119–127, Academic Press, New York.
- [15] Essig, A. and Caplan, S.R. (1989) Water movement: does thermodynamic interpretation distort reality? *Am. J. Physiol.* 256, C694–C698.
- [16] Finkelstein, A. (1989) Water movement: does thermodynamic interpretation distort reality? *Am. J. Physiol.* 256, C699.
- [17] Hammel, H.T. (1979) Forum on osmosis. I. Osmosis: diminished solvent activity or enhanced solvent tension? *Am. J. Physiol.* 237, R95–R107.
- [18] Hammel, H.T. (1979) Forum on osmosis. V. Epilogue. *Am. J. Physiol.* 237, R123–R125.
- [19] Soodak, H. and Iberall, A. (1979) Forum on osmosis. IV. More on osmosis and diffusion. *Am. J. Physiol.* 237, R114–R122.
- [20] Mauro, A. (1979) Forum on osmosis. III. Comments on Hammel and Scholander’s solvent tension theory and its application to the phenomenon of osmotic flow. *Am. J. Physiol.* 237, R110–R113.

- [21] Hildebrand, J.H. (1979) Forum on osmosis. II. A criticism of "solvent tension" in osmosis. *Am. J. Physiol.* 237, R108–R109.
- [22] Kleinhans, F.W. (1998) Membrane permeability modeling: Kedem-Katchalsky vs a two-parameter formalism. *Cryobiology* 37, 271–289.
- [23] Benga, G. (2003) Birth of water channel proteins – the aquaporins. *Cell Biol. Int.* 27, 701–709.
- [24] Stein, W.D. and Danielli, J.F. (1956) Structure and function in red cell permeability. *Discuss. Faraday Soc.* 21, 238–251.
- [25] Finkelstein, A. and Anderson, O.S. (1981) The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport. *J. Membr. Biol.* 59, 155–171.
- [26] Ussing, H.H. (1965) Transport of electrolytes and water across epithelia. *Harvey Lect.* 59, 1–30.
- [27] Rich, G.T., Shaafi, I., Romualdez, A. and Solomon, A.K. (1968) Effect of osmolality on the hydraulic permeability coefficient of red cells. *J. Gen. Physiol.* 52, 941–954.
- [28] Dainty, J. and House, C.R. (1966) An examination of the evidence for membrane pores in frog skin. *J. Physiol.* 185, 172–184.
- [29] Parisi, M. and Bourguet, J. (1985) Water channels in animal cells: a widespread structure? *Biol. Cell* 55, 155–157.
- [30] Sidel, V.W. and Solomon, A.K. (1957) Entrance of water into human red cells under an osmotic pressure gradient. *J. Gen. Physiol.* 41, 243–257.
- [31] Paganelli, C.V. and Solomon, A.K. (1957) The rate of exchange of tritiated water across the human red cell membrane. *J. Gen. Physiol.* 41, 259–277.
- [32] Macey, R.I. and Farmer, R.E. (1970) Inhibition of water and solute permeability in human red cells. *Biochim. Biophys. Acta* 211, 104–106.
- [33] Benga, G., Popescu, O., Borza, V., Pop, V.I., Muresan, A., Mocsy, I., Brain, A. and Wrigglesworth, J.M. (1986) Water permeability in human erythrocytes: identification of membrane proteins involved in water transport. *Eur. J. Cell Biol.* 41, 252–262.
- [34] Benga, G., Popescu, O., Pop, V.I. and Holmes, R.P. (1986) p-(Chloromercuri)benzenesulfonate binding by membrane proteins and the inhibition of water transport in human erythrocytes. *Biochemistry* 25, 1535–1538.
- [35] Parisi, M., Montoreano, R., Chevalier, J. and Bourguet, J. (1981) Cellular pH and water permeability control in frog urinary bladder. A possible action on the water pathway. *Biochem. Biophys. Acta* 648, 267–274.
- [36] Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.T., Bligny, R. and Maurel, C. (2003) Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* 425, 393–397.
- [37] Alleva, K., Niemietz, C.M., Sutka, M., Maurel, C., Parisi, M., Tyerman, S.D. and Amodeo, G. (2006) Plasma membrane of *Beta vulgaris* storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. *J. Exp. Bot.* 57, 609–621.
- [38] Zhang, R., Logee, K.A. and Verkman, A.S. (1990) Expression of mRNA coding for kidney and red cell water channels in *Xenopus oocytes*. *J. Biol. Chem.* 265, 15375–15378.
- [39] Parisi, M., Dorr, R.A., Ozu, M. and Toriano, R. (2007) From membrane pores to aquaporins: 50 years measuring water fluxes. *J. Biol. Phys.* 33, 5–6.
- [40] Pao, G.M., Wu, L.F., Johnson, K.D., Höfte, H., Chrispeels, M.J., Sweet, G. and Sandal, N.N. (1991) Evolution of the MIP family of integral membrane transport proteins. *Mol. Microbiol.* 5, 33–37.
- [41] Gorin, M.B., Yancey, S.B., Cline, J., Revel, J.P. and Horwitz, J. (1984) The major intrinsic protein (MIP) of the bovine lens fiber membrane: characterization and structure based on cDNA cloning. *Cell* 39, 49–59.
- [42] Sandal, N.N. and Marcker, K.A. (1988) Soybean nodulin 26 is homologous to the major intrinsic protein of the bovine lens fiber membrane. *Nucleic Acids Res.* 19, 9347.
- [43] Muramatsu, S. and Mizuno, T. (1989) Nucleotide sequence of the region encompassing the glpKF operon and its upstream region containing a bent DNA sequence of *Escherichia coli*. *Nucleic Acids Res.* 17, 4378.
- [44] Johnson, K.D., Höfte, H. and Chrispeels, M.J. (1990) An intrinsic tonoplast protein of protein storage vacuoles in seeds is structurally related to a bacterial solute transporter (GlpF). *Plant Cell* 6, 525–532.
- [45] Rao, Y., Jan, L.Y. and Jan, Y.N. (1990) Similarity of the product of the *Drosophila* neurogenic gene big brain to transmembrane channel proteins. *Nature* 345, 163–167.
- [46] Smith, C.P. and Chater, K.F. (1998) Cloning and transcription analysis of the entire glycerol utilization (gluABX) operon of *Streptomyces coelicolor* A3 (2) and identification of a closely associated transcription unit. *Mol. Gen. Genet.* 211, 129–137.
- [47] Baker, M.E. and Saier Jr., M.H. (1990) A common ancestor for bovine lens fiber major intrinsic protein, soybean nodulin-26 protein, and *E. coli* glycerol facilitator. *Cell* 26, 185–186.
- [48] Preston, G.M. and Agre, P. (1991) Isolation of the cDNA for erythrocyte integral membrane protein of 28 kD: member of an ancient channel family. *Proc. Natl. Acad. Sci. USA* 88, 11110–11114.
- [49] Agre, P. (2006) The aquaporin water channels. *Proc. Am. Thorac. Soc.* 3, 5–13.
- [50] Agre, P. (2009) Interview: A Conversation with Peter Agre. By Claudia Dreifus. The New York Times.
- [51] Preston, G.M., Carroll, T.P., Guggino, W.B. and Agre, P. (1992) Appearance of water channels in *Xenopus oocytes* expressing red cell CHIP28 protein. *Science* 256, 385–387.
- [52] Agre, P., Preston, G.M., Smith, B.L., Jung, J.S., Raina, S., Moon, C., Guggino, W.B. and Nielsen, S. (1993) Aquaporin CHIP: the archetypal molecular water channel. *Am. J. Physiol.* 265, 463–476.
- [53] Verkman, A.S. and Mitra, A.K. (2000) Structure and function of aquaporin water channels. *Am. J. Physiol. Renal Physiol.* 278, F13–F28.
- [54] Johanson, U. and Gustavsson, S. (2002) A new subfamily of major intrinsic proteins in plants. *Mol. Biol. Evol.* 19, 456–461.
- [55] Wallace, I.S. and Roberts, D.M. (2004) Homology modeling of representative subfamilies of Arabidopsis major intrinsic proteins. Classification based on the aromatic/arginine selectivity filter. *Plant Phys.* 135, 1059–1068.
- [56] Wudick, M.M., Luu, D.T. and Maurel, C. (2009) A look inside: localization patterns and functions of intracellular plant aquaporins. *New Phytol.* 184, 289–302.
- [57] Finn, R.N. and Cerdà, J. (2011) Aquaporin evolution in fishes. *Front Physiol.* 2, 44.
- [58] Soto, G., Alleva, K., Amodeo, G., Muschietti, J. and Ayub, N. (in press) New insight into the evolution of aquaporins from flowering plants and vertebrates: orthologous identification and functional transfer is possible. *Gene*. doi:<http://dx.doi.org/10.1016/j.gene.2012.04.021>.
- [59] Murata, K., Mitsuoka, K., Hirai, T., Walz, T., Agre, P., Heymann, J.B., Engel, A. and Fujiyoshi, Y. (2000) Structural determinants of water permeation through aquaporin-1. *Nature* 407, 599–605.
- [60] Fu, D., Libson, A., Miercke, L.J.W., Weitzman, C., Nollert, P., Krucinski, J. and Stroud, R.M. (2000) Structure of a glycerol conducting channel and the basis for its selectivity. *Science* 290, 481–486.
- [61] Sui, H., Han, B., Lee, J., Walian, P. and Jap, B. (2001) Structural basis of water-specific transport through the AQP1 water channel. *Nature* 414, 872–878.
- [62] Tajkhorshid, E., Nollert, P., Jensen, M., Miercke, L.O., O'Connell, J., Stroud, R. and Schulten, K. (2002) Control of the selectivity of the aquaporin water channel family by global orientational tuning. *Science* 296, 525–530.
- [63] de Groot, B.L., Frigato, T., Helms, V. and Grubmüller, H. (2003) The mechanism of proton exclusion in the aquaporin-1 water channel. *J. Mol. Biol.* 333, 279–293.
- [64] Hub, J.S., Grubmüller, H. and de Groot, B.L. (2009) Dynamics and energetics of permeation through aquaporins. What do we learn from molecular dynamics simulations? *Handb. Exp. Pharmacol.* 190, 57–76.
- [65] Hashido, M., Kidera, A. and Ikeguchi, M. (2007) Water transport in aquaporins: osmotic permeability matrix analysis of molecular dynamics simulations. *Biophys. J.* 93, 373–385.
- [66] Chara, O., McCarthy, A.N., Ferrara, C.G., Caffarena, E.R. and Grigera, J.R. (2009) Water behavior in the neighborhood of hydrophilic and hydrophobic membranes: lessons from molecular dynamics simulations. *Physica A* 388, 4551–4559.
- [67] Gerbeau, P., Amodeo, G., Henzler, T., Santoni, V., Ripoche, P. and Maurel, C. (2002) The water permeability of Arabidopsis plasma membrane is regulated by divalent cations and pH. *Plant J.* 1, 71–81.
- [68] Németh-Cahalan, K.L., Kalman, K. and Hall, J.E. (2004) Molecular basis of pH and Ca<sup>2+</sup> regulation of aquaporin water permeability. *J. Gen. Physiol.* 123, 573–580.
- [69] Alleva, K., Marquez, M., Villarreal, N., Mut, P., Bustamante, C., Bellati, J., Martínez, G., Civello, M. and Amodeo, G. (2010) Cloning, functional characterization, and co-expression studies of a novel aquaporin (FaPIP2;1) of strawberry fruit. *J. Exp. Bot.* 61, 3935–3945.
- [70] Bellati, J., Alleva, K., Soto, G., Vitali, V., Jozefkiewicz, C. and Amodeo, G. (2010) Intracellular pH sensing is altered by plasma membrane PIP aquaporin co-expression. *Plant Mol. Biol.* 74, 105–118.
- [71] Johansson, I., Larsson, C., Ek, B. and Kjellbom, P. (1996) The major integral proteins of spinach leaf plasma membranes are putative aquaporins and are phosphorylated in response to Ca<sup>2+</sup> and apoplastic water potential. *Plant Cell* 8, 1181–1191.
- [72] Johansson, I., Karlsson, M., Shukla, V.K., Chrispeels, M.J., Larsson, C. and Kjellbom, P. (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *Plant Cell* 10, 451–460.
- [73] Guenther, J.F., Chanmanivone, N., Galetovic, M.P., Wallace, I.S., Cobb, J.A. and Roberts, D.M. (2003) Phosphorylation of soybean Nodulin 26 on serine 262 enhances water permeability and is regulated developmentally and by osmotic signals. *Plant Cell* 15, 981–991.
- [74] Azad, A.K., Sawa, Y., Ishikawa, T. and Shibata, H. (2004) Characterization of protein phosphatase 2A acting on phosphorylated plasma membrane aquaporin of tulip petals. *Biosci. Biotechnol. Biochem.* 68, 1170–1174.
- [75] Zhang, R., van Hoek, A.N., Biwersi, J. and Verkman, A.S. (1993) A point mutation at cysteine 189 blocks the water permeability of rat kidney water channel CHIP28k. *Biochemistry* 32, 2938–2941.
- [76] Németh-Cahalan, K.L., Kalman, K., Froger, A. and Hall, J.E. (2007) Zinc modulation of water permeability reveals that aquaporin 0 functions as a cooperative tetramer. *J. Gen. Physiol.* 5, 457–464.
- [77] Yukutake, Y., Hirano, Y., Suematsu, M. and Yasui, M. (2009) Rapid and reversible inhibition of aquaporin-4 by zinc. *Biochemistry* 48, 12059–12061.
- [78] Hedfalk, K., Törnroth-Horsefield, S., Nyblom, M., Johanson, U., Kjellbom, P. and Neutze, R. (2006) Aquaporin gating. *Curr. Opin. Struct. Biol.* 16, 447–456.
- [79] Petrovic, M.M., Vales, K., Stojan, G., Basta-Jovanović, G. and Mitrović, D.M. (2006) Regulation of selectivity and translocation of aquaporins: an update. *Folia Biol.* 52, 173–180.
- [80] Conner, M.T., Conner, A.C., Bland, C.E., Taylor, L.H., Brown, J.E., Parri, H.R. and Bill, R.M. (2012) Rapid aquaporin translocation regulates cellular water flow: the mechanism of hypotonicity-induced sub-cellular localization of the aquaporin 1 water channel. *J. Biol. Chem.* 287, 11516–11525.
- [81] Maurel, C., Verdoucq, L., Luu, D.-T. and Santoni, V. (2008) Plant aquaporins: membrane channels with multiple integrated functions. *Annu. Rev. Plant Biol.* 59, 595–624.

- [82] Fetter, K., Wilder, V.V., Moshelion, M. and Chaumont, F. (2004) Interactions between plasma membrane aquaporins modulate their water channel activity. *Plant Cell* 1, 215–228.
- [83] Zelazny, E., Borst, J.W., Muylaert, M., Batoko, H., Hemminga, M.A. and Chaumont, F. (2007) FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. *Proc. Natl. Acad. Sci. USA* 104, 12359–12364.
- [84] Luu, D.T., Martinière, A., Sorieul, M., Runions, J. and Maurel, C. (2012) Fluorescence recovery after photobleaching reveals high cycling dynamics of plasma membrane aquaporins in Arabidopsis roots under salt stress. *Plant J.* 69, 894–905.
- [85] Beitz, E. (Ed.) (2009) Aquaporins. *Handbook of Experimental Pharmacology*. In: Universität Kiel Pharmazeutisches Inst. Gutenbergstr, Springer-Verlag, Berlin.
- [86] Ford, P., Rivarola, V., Chara, O., Blot-Chabaud, M., Cluzeaud, F., Farman, N., Parisi, M. and Capurro, C. (2005) Volume regulation in cortical collecting duct cells: role of AQP2. *Biol. Cell* 97, 687–697.
- [87] Marinelli, R., Tietz, P.S., Caride, A.J., Huang, B.Q. and LaRusso, N.F. (2003) Water transporting properties of hepatocyte basolateral and canalicular plasma membrane domains. *J. Biol. Chem.* 278, 43157–43162.
- [88] Calamita, G., Gena, P., Ferri, D., Rosito, A., Rojek, A., Nielsen, S., Marinelli, R.A., Frühbeck, G. and Svelto, M. (2012) Biophysical assessment of aquaporin-9 as principal facilitative pathway in mouse liver import of glucogenetic glycerol. *Biol. Cell*. doi:<http://dx.doi.org/10.1111/boc.20110006>.
- [89] Saadoun, S., Papadopoulos, M.C., Hara-Chikuma, M. and Verkman, A.S. (2005) Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. *Nature* 434, 786–792.
- [90] Papadopoulos, M.C., Saadoun, S. and Verkman, A.S. (2008) Aquaporins and cell migration. *Pflugers Arch.* 456, 693–700.
- [91] Manley, G.T., Fujimura, M., Ma, T., Noshita, N., Filiz, F., Bolln, A.W., Chan, P. and Verkman, A.S. (2000) Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nature Med.* 6, 159–163.
- [92] Maurel, C. (2007) Plant aquaporins: novel functions and regulation properties. *FEBS Lett.* 581 (12), 2227–2236.
- [93] Martre, P., Morillon, R., Barrieu, F., North, G.B., Nobel, P.S. and Chrispeels, M.J. (2002) Plasma membrane aquaporins play a significant role during recovery from water deficit. *Plant Phys.* 130, 2101–2110.
- [94] Siefritz, F., Tyree, M.T., Lovisolo, C., Schubert, A. and Kaldenhoff, R. (2002) PIP1 plasma membrane aquaporins in tobacco: from cellular effects to function in plants. *Plant Cell* 14, 869–876.
- [95] Javot, H., Lauvergeat, V., Santoni, V., Martin-Laurent, F., Güçlü, J., Vinh, J., Heyes, J., Franck, K.I., Schäffner, A.R., Bouchez, D. and Maurel, C. (2003) Role of a single aquaporin isoform in root water uptake. *Plant Cell* 15, 509–522.
- [96] Moshelion, M., Becker, D., Biela, A., Uehlein, N., Hedrich, R., Otto, B., Levi, H., Moran, N. and Kaldenhoff, R. (2002) Plasma membrane aquaporins in the motor cells of *Samanea saman*: diurnal and circadian regulation. *Plant Cell* 3, 727–739.
- [97] Vander Willigen, C., Postaire, O., Tournaire-Roux, C., Boursiac, Y. and Maurel, C. (2006) Expression and inhibition of aquaporins in germinating Arabidopsis seeds. *Plant Cell Physiol.* 47, 1241–1250.
- [98] Soto, G., Alleva, K., Mazzella, M.A., Amodeo, G. and Muschietti, J.P. (2008) AtTIP1;3 and AtTIP5;1, the only highly expressed Arabidopsis pollen-specific aquaporins, transport water and urea. *FEBS Lett.* 582, 4077–4082.
- [99] Mut, P., Bustamante, C., Martínez, G., Alleva, K., Sutka, M., Civello, M. and Amodeo, G. (2008) A fruit-specific plasma membrane aquaporin subtype PIP1;1 is regulated during strawberry (*Fragaria x ananassa*) fruit ripening. *Physiol Plant.* 132, 538–551.
- [100] Ehlert, C., Maurel, C., Tardieu, F. and Simonneau, T. (2009) Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Phys.* 150, 1093–1104.
- [101] Maurel, C., Simonneau, T. and Sutkam, M. (2010) The significance of roots as hydraulic rheostats. *J. Exp. Bot.* 61, 3191–3198.
- [102] Takano, J., Motoko Wada, M., Ludewig, U., Schaaf, G., von Wiren, N. and Takano, T.F. (2006) The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *Plant Cell* 18, 1498–1509.
- [103] Calamita, G. (2005) Aquaporins: highways for cells to recycle water with the outside world. *Biol. Cell* 97, 351–353 [Reproduced with permission© Portland Press Ltd].
- [104] MacAulay, N., Hamann, S. and Zeuthen, T. (2004) Water transport in the brain: role of cotransporters. *Neuroscience* 129, 1031–1044.
- [105] Fischbarg, J., Kunyan, K., Vera, J.C., Arant, S., Silverstein, S., Loike, J. and Rosen, O.M. (1990) Glucose transporters serve as water channels. *Proc. Natl. Acad. Sci. USA* 87, 3244–3324.
- [106] Chara, O., Ford, P., Rivarola, V., Parisi, M. and Capurro, C. (2005) Asymmetry in the osmotic response of a rat cortical collecting duct cell line: role of aquaporin-2. *J. Membr. Biol.* 207, 143–150.
- [107] Kiil, F. (1982) Mechanism of osmosis. *Kidney Int.* 21, 303–308.
- [108] Kargol, M. and Kargol, A. (2003) Mechanistic formalism for membrane transport generated by osmotic and mechanical pressure. *Gen. Physiol. Biophys.* 22, 51–68.
- [109] Raghunathan, A. and Aluru, N. (2006) Molecular understanding of osmosis in semipermeable membranes. *Phys. Rev. Lett.* 97, 1–4.
- [110] Curry, M.R., Shachar-Hill, B. and Hill, A.E. (2001) Single water channels of aquaporin-1 do not obey the Kedem–Katchalsky equations. *J. Membr. Biol.* 181, 115–123.
- [111] Hill, A.E., Shachar-Hill, B. and Shachar-Hill, Y. (2004) What are aquaporins for? *J. Membr. Biol.* 197, 1–32.
- [112] van 't Hoff, J.H. (1966) Osmotic Pressure and Chemical Equilibrium. From Nobel Lectures, Chemistry, 1901–1921. Elsevier, Amsterdam.
- [113] Agre, P. (2008) In: Ahlberg, P. (Ed.) Nobel Lectures in Chemistry, 2001–2005. Göteborg University, Sweden.